An Investigation of Sugar Feeding by Black Flies (Diptera: Simuliidae)

by

Steve G. Burgin, B. Sc.

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Brock University
St Catharines, Ontario

Abstract

Although much research has been conducted on blood-meal acquisition in adult female black flies (Diptera: Simuliidae), the same cannot be said for sugar-meals. Both sexes feed on sugar which provides energy for flight and it has been commonly held that nectar is the major carbohydrate source. This thesis addresses the question of whether a non-floral carbohydrate source, specifically homopteran honeydew, is ingested by male and female black flies. Black flies reared in the laboratory have been observed to readily ingest freshly excreted and older (dry) honeydew when presented with honeydew coated tamarack branches. Field work was conducted in Algonquin Park, Ontario in the spring and summer of 1993. Three separate studies were designed to test whether homopteran honeydew is an important carbohydrate source for black flies and whether flies from different habitats utilize different sugar sources. The sugars melezitose and / or stachyose are known to occur in a variety of homopteran honeydews and therefore were used as indicators of honeydew feeding by black flies.

In the first study, black flies were collected with insect nets from a stand of *Larix larcina* heavily infested with honeydew - producing homopterans (*Adelges lariciatus*). Six black fly species were captured: *Simulium venustum*, *S. rostratum*, *S. vittatum*, *Stegopterna mutata*, *S. aureum* and *S. quebecense*. Samples of honeydew and individual black flies were tested using thin layer chromatography (T. L. C.) with fructose, glucose, sucrose, turanose, melezitose, raffinose and stachyose as standards. All sugars except turanose and melezitose were found in the adelgid honeydew samples. Since the sugar melezitose was absent from honeydew samples, stachyose was used to indicate that black flies were feeding from this particular honeydew source. Of the 201 black flies tested, 194 contained sugars which occurred in 16 combinations. Stachyose combinations excluding melezitose, present in 45.9 % of flies, were used to indicate that black flies had been feeding on the adelgid honeydew.

In the second study, black flies were collected in the morning and evening on 8 collection dates, using a vehicle mounted insect net. The crops and midguts of 10 male and 10 female *Simulium venustum* were dissected on each sample date. In total the gut contents of 320 individual flies were analysed by T. L. C. The sugars identified from these flies were present in the following proportions: fructose (100.0%), glucose (100.0%), sucrose/turanose (50.4%), melezitose (30.3%), raffinose (18.8%) and stachyose (8.7%). These sugars occurred in fourteen different combinations. It is argued that the presence of melezitose and / or stachyose indicates that black flies had fed on homopteran honeydew. Significantly more female flies (40.0%) than male flies (27.5%) had fed on honeydew.

In the third study, adult black flies were sampled by sweep netting vegetation in four habitats in the morning and evening on 8 collection dates. The habitats are as follows: (1) Davies Bog, (2) Abandoned Air Field (dominated by blueberries, *Vaccinium* spp.), (3) Deciduous Habitat and (4) Coniferous Habitat. Sugars in the crops and midguts of female flies were tested by T. L. C. and, for *S. venustum,* it was found that significantly fewer flies (18.8%) from the Air Field contained honeydew than from the other three sites (Davies Bog, 34.4%; Deciduous Habitat, 36.2%; Coniferous Habitat, 25.0%).

Of the 1287 black flies tested individually by T. L. C. 441 (34.3%) contained melezitose and / or stachyose sugars indicating that this proportion of the population were feeding from Homopteran honeydew. It is therefore clear that floral (nectar) sugars are not the only source of carbohydrates available to black flies.

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Introduction

Larval Biology

Depending on the species, the immature stages (larvae and pupae) of black flies are found within rivers, streams or trickles with current velocities ranging from rapid to imperceptibly slow (Burger, 1987). The larvae of all species of black fly require a constant flow of water (even a thin film) over their bodies to supply both oxygen (which diffuses through the larval cuticle), and particulate nutrients which are filtered from the current using the larval labral fans. Exceptions to this are the "obligate scrapers", such as *Twinnia spp.* Stone and Jamnback and *Gymnopias spp.* Stone, which do not possess labral fans for "filter feeding" but instead use their mandibles to scrape algae (Currie and Craig, 1987). In order to remain in areas of optimal flow, larvae attach themselves to the substrate with the aid of salivary gland"silk", in which the posterior ring of larval hooklets is embedded. Larvae pass through approximately 5-7 instars (a period of about 10 days to several months if the larvae over-winter) before pupating, at which time a slightly different form of "silk" is used by the pharate pupa to construct its own cocoon (Hinton, 1958).

Pupal Biology

The pupae which are immobile (firmly attached to natural or artificial substrates) are not at risk when water levels fluctuate, as the pupal cocoon and cuticle protect the developing adult against desiccation. In addition, oxygen uptake (gas exchange) does not appear to be affected after removal from water (Crosskey, 1990). Feeding does not occur in the pupal stage so subsequent energy requirements of the pupae and newly emerged adults must come from nutrient stores accumulated in the larval stage (Magnarelli and Burger, 1984). After approximately a week of pupal development the adult black fly emerges from the

pupal exuvium in a bubble of gas and is conveyed to the water surface (Davies *et al.,* 1962). There is some evidence that black flies tend to emerge in the morning more frequently than at any other time of day or night (Wenk, 1981).

Adult Biology

In most species of black fly, the adult female requires a blood meal for the development of eggs. For some species, humans can serve as the blood-host. This aspect of black fly biology has resulted in a great deal of research directed towards the control of the notoriously vicious biting species, and those species which serve as vectors of disease. Perhaps the most studied black flies are members of the *Simulium damnosum* Theobald, 1903 (complex) which are vectors of the filarial worm *Onchocerca volvulus* which causes the condition, in humans, known as onchocerciasis or river blindness. This parasite, found in areas of Africa and Central and tropical South America, was estimated by the World Health Organization (1987) to infect at least 17.5 million people (Crosskey, 1990). Although there are no human parasites or diseases transmitted by black flies in Canada, they are still considered to be important insect pests. In Saskatchewan and Alberta outbreaks of the black fly *Simulium arcticum* Malloch, resulted in the death of many cattle due to anaphylactic shock (Charnetski and Haufe, 1981). Other species in Canada are reported to be vectors of *Leucocytozoon spp.* which cause a malaria-like condition in birds (Fallis, 1980).

According to Davies and Györkös (1990), female black flies may be placed into one of three categories which indicate the basic source of nutrient energy used to develop eggs: (1) obligate autogeny (development of eggs using energy accumulated in the larval stage), (2) facultative autogeny for the first ovarian cycle only and (3) obligate anautogeny (development of eggs using energy derived from blood-meals). A fourth category, facultative anautogeny, is mentioned by Anderson

(1987) along with facultative autogeny to describe the species *S. vittatum* and *S. decorum* which may or may not blood feed in the first ovarian cycle depending on the nutrients available to the larvae. The term "primaparous autogeny" is used by Crosskey (1990) in the same sense as "facultative autogeny in the first ovarian cycle" of Davies and Györkös (1990) and the two separate classifications of Anderson (1987).

Obligate Autogeny

Few species of black fly (at least three) found in Algonquin Park are considered to be obligatorily autogenous (Appendix 1): *T. tibblesi, Helodon gibsoni* and *Cnephia dacotensis* (Davies and Györkös, 1990). The sequence of events between emergence and oviposition are well known for *C. dacotensis*, a "ground mating" species (Davies and Peterson, 1956; Crosskey, 1990). Females of this species are univoltine (i.e., have a single ovarian cycle per generation), mate and oviposit within a few hours of emergence and then die (Davies and Peterson, 1956; Anderson, 1987; Crosskey, 1990). There is no need for additional nutrients (apart from stored nutrients) as a result of this reproductive strategy and apparently, *C.dacotensis* does not require a nectar meal during its short life (Downes, 1958). The life cycle of "ground mating" black flies such as *Gymnopais spp.* and *Crozetia sp.,* which inhabit subarctic and subantarctic regions, is apparently an adaptation to the harsh environmental conditions such as short season, cold weather and decreased food availability (Davies and Györkös, 1990; Crosskey, 1990).

Facultative Autogeny

There are at least seven facultatively autogenous species, for the first gonotropic cycle, in Algonquin Park (Appendix 1), including: *Prosimulium fontanum*, *P. fuscum*, *P. multidentatum*, *Stegopterna mutata*, *Simulium vittatum*, *S. longistylatum*, *S. decorum*, and possibly *Eusimulium excisum* (Davies *et al.,* 1962; Davies and Györkös, 1990). In these species, energy for egg development may be carried over from the immature stages. However, as these species are multivoltine (i.e., have more than one ovarian cycle per generation) the necessary nutrients for subsequent egg development may require blood and / or sugar meals (Magnarelli and Burger, 1984).

The mandibles of facultatively autogenous and obligatorily anautogenous females are well developed and finely serrate and are, therefore, suitable for obtaining blood meals. This differs markedly from obligatorily autogenous species such as *C. dacotensis* in which the mandibles of the female (and male) are atrophied and appear as thin irregularly shaped plates. The mandibles of males, in all species of black fly, are reduced as this sex does not blood feed (Crosskey, 1990).

Obligate Anautogeny

At least 24 species of black fly in Algonquin Park are considered to be anautogenous (Appendix 1), a few of which are: *Prosimulium mixtum*, *Ectemnia invenusta*, *Eusimulium aureum*, *Eusimulium vernum*, *Eusimulium quebecense*, *Eusimulim croxtoni*, *Eusimulium emarginatum*, *Eusimulium euryadminiculum*, *Simulium parnasum*, *Simulium tuberosum*, *Simulium venustum*, *Simulium rostratum* (Davies *et al.,* 1962; Davies and Györkös, 1990). The behaviour patterns of anautogenous species are perhaps the most complex and least

understood of the three categories. Anautogenous black flies perform a number of energetically expensive behaviours during their adult life, a summary of which was given by Anderson (1987).

Flight

The formation of mating swarms is perhaps the most energetically expensive activity of male black flies (Davies and Peterson, 1956; Davies *et al.,* 1962). Female black flies expend energy in flight while searching for a mate, for blood-host(s) and for suitable oviposition site(s). Both sexes must expend energy in order to find sources of sugar to fuel flight (Crosskey, 1990). A number of meteorological factors have been found to affect flying activities of black flies. The most important factors are considered to be light level and temperature, although wind speed and relative humidity, may be of some importance (McCreadie *et al.,* 1986; Crosskey, 1990).

Carbohydrate Requirements

It is apparent from the literature that many species of black fly require carbohydrates in order to perform behaviours directly associated with reproduction. Consequently, the ability of female black flies to harass or serve as vectors of parasites to wildlife, domestic animals and humans may be affected by their ability to obtain and utilize carbohydrates for flight. It may be pertinent to note that the ability of sand flies (Diptera: Psychodidae) to vector the parasite *Leishmania* spp. in the laboratory appears to be affected by the sugar meals provided (Schlein and Warburg, 1986). The sugar - feeding behaviour of both males and females has received little attention to date.

Due to the collection methods used in many studies (i.e., CO_2 baited traps or sweep nets of flies attracted to the collectors), female flies have been tested for the presence of sugar more often than have male flies (Lewis and Domoney, 1966; Brenner and Cupp, 1980; Walsh and Garms, 1980). Although no numbers were given, Hunter (1977) reported that almost all male flies captured from mating swarms tested positive for fructose or sugars containing fructose with cold anthrone reagent. It has been suggested that male flies visit flowers more frequently than female flies (Smart, 1943; Wenk, 1965).

The energy for flight comes from sugar-meals (i.e., carbohydrates), whereas blood meals (i.e., protein) are used in the development of eggs (Hocking, 1953; Davies *et al.,* 1962; Sutcliffe, 1986; Crosskey, 1990). As stated by Anderson (1987) nectar feeding in females may occur before and a number of times after mating. Sutcliffe (1986) indicates that mating may occur before sugar feeding, a claim supported by Wenk (1965). However, observations by Hunter (1979) of the species *Austrosimulium pestilens* Mackerras and Mackerras demonstrate that sugar-feeding can occur before mating. Since female black flies attracted to a blood-host are found with sperm in the spermatheca, it is now generally accepted that black flies mate prior to host-seeking (Brenner and Cupp, 1980; Laird, 1981; Sutcliffe, 1986). In the study by Brenner and Cupp (1980) all females attracted to the collectors had mated previously and 117 / 124 (94%) of these flies contained sugar, suggesting that sugar feeding occurs before host seeking. Blood-fed and gravid females were found on plants in the study by Wenk (1965) which suggests that, after blood-feeding, females may rest on plants which are potential sources of nectar. Crosskey (1990) states that gravid flies take nectar (i.e., have full crops or are visiting flowers) in order to provide energy for the oviposition flight. By examining the fat body content and ovaries (for relict eggs) it has also been found that parous flies (i.e., those which have oviposited previously) also contain sugar (Brenner and Cupp, 1980; Walsh and Garms, 1980). This indicates that a female

black fly may consume sugar meals several times during her life. Alternatively, Brenner and Cupp (1980) suggest that black flies may simply metabolize sugars slowly and hence may test positive for sugars a relatively long time after feeding.

Floral Associations

Research on the subject of sugar feeding in black flies has been conducted with the perception that flower nectar is the main source of carbohydrates (Lewis and Domoney, 1966; Disney, 1970; Hunter, 1977; Watanabe, 1977; Cupp and Collins, 1979; Brenner and Cupp, 1980; Walsh and Garms 1980). This is understandable, as there have been several observations of black flies visiting flowers (Müller, 1873, *In* Hocking 1953; Knuth 1906-1909; Robertson, 1928; Smart, 1943; Hocking, 1953; Hocking and Pickering, 1954; Davies and Peterson, 1956; Wenk, 1965; Proctor and Yeo, 1972; Kearns, 1992; see Appendix 2).

It has been suggested that black flies have a preference for certain flowers and that they use olfactory cues to locate nectar sources, as the flowers visited are generally small with inconspicuous yellow - green colouration (Wenk, 1965).

Observations of large numbers of black flies visiting flowers were recorded on two occasions. In Algonquin Park, Ontario, Davies and Peterson (1956) collected 235 female and 3 male black flies, mostly *S. venustum*, from sweep net samples around blueberries. In England, Smart (1943) captured well over 500 black flies (6 to 1 ratio of males to females) from a patch of ivy flowers during one collecting session. Studies by Müller (1873), Knuth (1906-1909), Robertson (1928), and Kearns (1992) were designed to survey all insects visiting flowers of various species. These surveys resulted in very few records of black flies on plants. For instance, Robertson (1928) recorded over 15000 insect visits to 441 species of plant, of which less than 10 visits were by simuliids. In the survey by Kearns (1992), over 1100 dipteran visitors (representing 138 species) were

collected from 66 species of plant, resulting in the capture of only one individual black fly. Several similar studies failed to record even one black fly from flowers (Drabble and Drabble, 1927; Finnamore and Neary, 1978; Kato *et al.,* 1990).

The first person to recognize the importance of insects in the pollination of blueberries was Phipps (1930) (Finnamore and Neary, 1978). Although he reported 18 species of Diptera from blueberries, Phipps (1930) did not believe that flies were of any importance in pollination (Finnamore and Neary, 1978). Finnamore and Neary (1978) consider several dipteran species to be potential or occasional pollinators of blueberries; however, black flies are not included among them (Appendix 3).

It has been observed that the flowering time of blueberries broadly overlaps with the peak of black fly activity within Algonquin Park, Ontario. Observations by Davies and Peterson (1956) from Algonquin Park suggest that black flies frequently visit blueberry flowers. In addition, numerous casual observations suggest that black flies occur in high numbers in areas where blueberries occur. It has been concluded from these observations that black flies visit blueberry flowers to obtain nectar which provides energy for flight. Furthermore, it has been suggested that the nectar feeding activity and extremely high numbers of black flies increases fruit formation in blueberries in Algonquin Park (Strickland and LeVay, 1986). It should be pointed out that, as a result of the collection methods in the study by Davies and Peterson (1956), black flies collected from around blueberry shrubs may have been more attracted to the collectors (as potential blood meal sources) than to the flowers. Based on personal observations, the association of black flies and blueberries does not appear as strong as has been suggested. In casual observations of blueberries, the black flies swarming about the researchers head sometimes landed on blueberry flowers, albeit briefly, but did not appear to enter the flowers before taking flight. Considering the large number of black flies in Algonquin Park, it is interesting that patches of flowering plants are never observed

to be crowded with black flies. It is an interesting fact, therefore, that the crops of a large proportion of both male and female black flies contain sugar meals.

Cold Anthrone Reagent

Earlier research in which the cold anthrone reagent was used, was conducted with mosquitoes (Van Handel 1967, and 1972). The information derived from this technique may be somewhat limited, as only fructose or fructose - containing sugars may be detected (Van Handel 1967). The presence of a clear refractive liquid in the crop (diverticulum) of black flies was noted by Lewis (1953) and Disney (1970). In subsequent studies it was confirmed, with the aid of the cold anthrone reagent, that the liquid contained fructose and / or sugars with a fructose component (Hunter, 1977; Cupp and Collins, 1979; Brenner and Cupp, 1980; Walsh and Garms 1980; McCreadie *et al.,* 1994). Sugar was detected in 65 - 94% of wild black flies (Walsh and Garms 1980; Brenner and Cupp, 1980; McCreadie *et al.,* 1994).

Chromatography

By using paper chromatography (Watanabe, 1977) and thin - layer chromatography (Lewis and Domoney, 1966), it was found that the crops of black flies may contain glucose, fructose, sucrose, maltose, melibiose and occasionally raffinose. Watanabe (1977) determined that most flies (81.6 %) in his study contained 4 or 5 sugars and that sucrose and raffinose occurred least frequently. Lewis and Domoney (1966) detected sugars in all 89 flies examined, in the following frequencies; glucose (100%), fructose (97.7%), sucrose (87.6%), maltose (46.1%), melibiose (20.2%) and occasionally raffinose (4.5%).

Crop

It is known that the crop has no digestive function (i.e., does not contain digestive enzymes) but instead serves as a storage organ for ingested sugars (Crosskey, 1990). No invertase activity has been detected in the crops of adult black flies (Yang and Davies, 1968). It has been stated that sugars and water will not move through crop tissue (Downes and Dahlem, 1987). Liquids entering and leaving the crop must, therefore, do so by the duct which attaches to the oesophagus, located anterior to the mid - gut.

The amount of liquid which can be contained in the crop of black flies was determined in three studies. Hocking (1953) found that the crops of *S. venustum* and *S. vittatum* can hold approximately 2.16 and 1.52 µl of sugar, respectively. Lewis and Domoney (1966) stated that *S. damnosum* can hold in excess of 0.25 µl. Watanabe (1977) determined that species in the genus *Simulium* contain less sugar (1.7 - 2.6 µl) than species in the genus *Prosimulium* (2.4 - 2.9 µl). The different crop capacities of these two genera are most likely due to the size differences, as *Prosimulium spp.* are generally larger (Crosskey, 1990).

According to Disney (1970), sugar meals of wild caught flies (i.e., older flies) will go directly into the mid - gut whereas in reared flies (i.e., younger flies) sugar meals will first be diverted into the crop. Cupp and Collins (1979) provide one of the only estimates of sugar meal digestion rates for black flies. By using the cold anthrone reagent to detect fructose or fructose - containing sugars, it was found that 40% of enclosed (i.e., inactive) flies contained fructose 2 days after a sugar meal. Hocking (1953) found that, on a diet of glucose (25 % solution) flight times of approximately 19 hours and 25 hours could be sustained by *S. venustum* and *S. vittatum*, respectively.

Pollen

Specimens of *S. damnosum* have been found with pollinia (pollen grain clusters) attached to their mouthparts (Lewis, 1953). In a study of black flies in Australia, Hunter (1977) found that 43 of 153 black flies had pollen in their crops. In this same study, pollen grains of *Pinus spp.* were found on the external surfaces of flies. The presence of pollen in the crops of black flies appears to provide further evidence of nectar - feeding activities (Hunter, 1977). However, care must be taken to determine if pollen adhering to external body parts, is from anemophilous (wind pollinated) plants which may not provide nectar to visiting insects. The record of pine pollen by Hunter (1977) is interesting in this respect. In addition, ingested pollen grains must also be carefully examined, as it might be possible for flies to ingest airborne pollen that had adhered to non - floral sources of sugar, such as honeydew.

Non - Floral Carbohydrates (Honeydew)

Honeydew is a syrupy liquid excreted as a waste product by insects of the order Homoptera such as aphids (Aphididae), coccids (Kermidae), white flies (Aleyrodidae) and leaf hoppers (Cicadellidae) (Gray and Fraenkel, 1954; Byrne and Miller, 1990). Members from each of these families can be found feeding on virtually all plants from trees to grasses (Borror, *et al.,* 1989). Many species cause a considerable amount of damage to agricultural crops as a result of feeding (i.e., damaging tissues by inserting stylets, gall formation, ingesting large amounts of sap and transmitting viruses), and reproduction (i.e., ovipositing) (Kennedy and Stroyan, 1959; Borror, *et al.,* 1989; Tarczynski *et al.,* 1992). The waste products of

these insects can also be damaging, as fungus can grow on leaf surfaces covered in honeydew (Byrne and Miller, 1990).

In a paper by Downes and Dahlem (1987) it is argued that the importance of honeydew to dipterans has been greatly underestimated. Seven main points are given in support of this theory. First it is stated that dipterans exhibit opportunistic sugar feeding behaviours and are, therefore, able to take advantage of this abundant carbohydrate source. A second behavioural argument relates to the erratic flight of dipterans, which allows them to detect "glints" of light reflected from honeydew coated surfaces. In addition, it is possible for flies to locate a sugar source based on odour cues (Dethier, 1976). Dipterans also appear to be physically adapted to detecting sugar coated surfaces once they have landed, as they have tarsal taste receptors. Another physical characteristic of dipterans is the pseudotracheate labellum which apparently allows flies to feed on dry sugar material with a reduced amount of water loss. The fifth point is based on information from the fossil record which suggests that feeding on homopteran honeydew may be the pleisiotypic condition in Diptera as homopterans were present in the Permian (approximately 250 MYBP) and the first dipterans appeared in the Triassic (approx. 240 MYBP). Therefore, both of these groups were present long before flowering plants which did not appear until the Cretaceous (135 MYBP) (Downes and Dahlem, 1987). The final two points relate to the abundance and composition of honeydew. These properties are of interest in this study and will be expanded upon.

Honeydew Abundance

Honeydew - excreting homopterans have a worldwide distribution. The habitats and positions within habitats occupied by different species varies considerably. Within woodlands, homopterans may be present in all levels of the

canopy and are even associated with the roots of plants. Populations of aphids can become extremely high in a relatively short time period (Dixon, 1973). Given optimal conditions (i.e., predators and competition for resources absent), a single aphid, maturing in 14 days, producing 30 offspring and with 9 generations per year, can produce 600 x 10^9 individuals (Dixon, 1973). It is estimated that densities can frequently reach 2.0 x 10^9 insects per acre (Dixon, 1973). Whitefly numbers on cotton plants (*Gossypium hirsutum* L.) have been found to exceed 100 per cm^2 (Tarczynski *et al.,* 1992). The rates of honeydew excretion for 5 species of aphid have been found to range from 0.04 µl -17.1µl / 10 hours, depending on a number of factors, such as: species, instar, temperature, wind, time of day (i.e., excretion is lowest before noon), and host plant (Auclair, 1963). When these factors are considered together (i.e., wide distribution, large populations and honeydew excretion rate) it is apparent that honeydew can be very abundant in the environment. Downes and Dahlem (1987) state that honeydew does not occur evenly over vegetation, but will appear as strictly localized patches on the upper surface of leaves below groups of actively feeding homopterans. However, it seems likely that honeydew falling from these higher locations may be dispersed by the action of wind. Precipitation (i.e., rain and dew) may further increase the dispersal of honeydew or may cause droplets to coalesce, forming more evenly coated surfaces.

Honeydew Composition

Honeydew - excreting homopterans feed by inserting their stylets into the phloem tissue of a host plant to withdraw fluid. A number of hypotheses have been developed to explain the apparently wasteful excretion of large amounts of honeydew. One generally accepted theory states that homopterans must ingest large amounts of phloem sap, containing mostly carbohydrates, to obtain amino

acids and / or other essential minerals which are in low concentrations (Kennedy and Stroyan, 1959; Byrne and Miller, 1990). It has been suggested that the presence of oligosaccharides in honeydew may have an osmoregulatory function (Fisher, *et al.,* 1984).

Many substances are now known to occur in the honeydews of various homopterans including several sugars, between 9 and 23 amino acids, citric acid, malic acid, ducitol, plant growth hormones, but little if any protein (Gray, 1952; Auclair, 1963). A number of studies have demonstrated that the phloem sap ingested by homopterans differs chemically from the honeydew excreted (Mittler, 1958; Hussain, *et al.,* 1974; Byrne and Miller, 1990; Tarczynski, *et al.,* 1992; see Appendix 4). However, it is known that the composition (including the carbohydrate components) of honeydew can vary with the species of insect and species of host plant being studied (Ewart and Metcalf, 1956; Auclair, 1963).

Amino Acids in Honeydew

A number of amino acids have been detected in honeydew but not the host plant. In coccid honeydew, tyrosine, histidine and three unknown amino acids were found (Srivastava and Varshney, 1966). In whitefly honeydew, aspartic acid, threonine, serine, asparagine, glutamic acid and glutamine were identified (Gray, 1952; Byrne and Miller, 1990).

Carbohydrates in Honeydew

The most commonly encountered sugars in honeydew are fructose, glucose, sucrose and the oligosaccharide melezitose (Mittler, 1958; Auclair, 1963). However, as many as 13 different sugars have been detected in honeydew. These carbohydrates may exceed 80% of the total weight of freshly excreted honeydew

(Ewart and Metcalf, 1956; MacVicker *et al.,* 1990). It has been found that the percentage composition of carbohydrates in honeydew does not change over time which suggests that metabolism does not take place once the honeydew is excreted (Byrne and Miller, 1990). Four of these sugars (erlose, melezitose, trehalulose and turanose) are thought to be unique to honeydew (Gray and Fraenkel, 1953; Belliardo *et al.*, 1979; Byrne and Miller, 1990; Moore *et al.*, 1987; see Appendix 5). Unfortunately, erlose and trehalulose are not commercially available.

Melezitose was first collected in 1859 from larch trees, from which the sugar's name is derived (meleze, French for larch) (Auclair,1963). It has now been accepted that melezitose is not a product of plants, but of the homopterans feeding from the plants (Bacon and Dickinson, 1955; Bacon and Dickinson, 1957; Mittler,1958; Kandler and Hopf, 1980; Byrne and Miller, 1990). The sugars melezitose and its hydrolysis product turanose can be positively identified and may, therefore, be used to indicate that flies have ingested honeydew. Melezitose has been identified from the honeydew of a number of homopterans.

The sugar stachyose has also been identified in the honeydew of homopterans (Byrne and Miller, 1990; Tarczynski *et al.,* 1992; Davis et al., 1993). This sugar is known to be a dominant sugar in the storage organs of a variety of plants, but may also be present as a transport sugar (Kandler and Hopf, 1980; Byrne and Miller, 1990).

It is known that the composition of honeydew can vary depending on the species of Homoptera as well as the species of host plant being studied (Ewart and Metcalf, 1956; Auclair, 1963). For this reason, it is possible for sugars such as melezitose or stachyose to be absent from honeydew (Hendrix *et al.,* 1992).

Sugar Feeding in Black Flies

It is now generally believed that dipteran insects obtain energy for flight from carbohydrates found in flower nectar (Crosskey, 1990). Much of the research into the subject of sugar feeding in black flies has been conducted with the perception that flower nectar is the main source of these sugars.

In the studies using chemical techniques to determine crop contents, it was indicated that the sugars present (i.e., fructose, glucose, sucrose, maltose, melibiose and raffinose) were obtained from plant nectar (Appendix 5). Further evidence of nectar feeding was provided by the observation of pollen in the crops of black flies and by observations of several species visiting flowers.

Alternative sources of sugar such as extrafloral nectaries, plant exudates (i.e., sap from wounds), fruit juices and Homopteran honeydew, are still largely ignored. In the recently published book by Crosskey (1990), extrafloral sources of sugar were considered to be of minor importance to black flies. The rationale for this belief seems to be that flower nectar is seldom in short supply. In addition very few observations of black flies feeding at these extrafloral sources have been reported. This is particularly true in the case of honeydew, as only one observation of honeydew - feeding has been reported for black flies (Crosskey, 1990). This behaviour may be infrequently observed for a number of reasons. First, it is evident that few researchers have actually looked for black flies feeding on honeydew. Second, even if attempts were made to observe black flies visiting honeydew, it is unlikely that significant numbers would be found, as honeydew covers a very large area and may be distributed vertically on the leaves of taller vegetation.

Secondly, honeydew is not found in easily observed patches (such as flowers) as homopterans are widespread among and within habitats. Within forests homopterans may be present in all levels of the canopy, further increasing the area covered by honeydew. Although many homopterans will form colonies, the

honeydew droplets can be dispersed as they fall through the canopy (Downes, 1974). As previously mentioned, rain, dew, wind and other factors may also cause further dispersal of honeydew. Based on personal observation, it has been found that in many areas within Algonquin Park, honeydew is abundant on understory plants and within the tree canopy on the upper surfaces of leaves (including needles).

In the laboratory, black flies have been observed feeding on leaves covered with a thin, clear coating of material, which was most likely honeydew (Pers. obs.). Several observations within the literature can be explained if black flies are assumed to be feeding on honeydew. A similar behaviour was described by Hunter (1977); however, in this case it was thought that the black flies were actually biting into the leaf tissue to extract fluid. Additional observations of black flies feeding from leaf tissue fluid and from extrafloral nectar have been recorded (Walsh, 1984, *In* Crosskey, 1990). The following quotation from Crosskey (1990) is interesting as the apparent contradiction between 'nectar' fed flies and the reduced number of nectar - producing plants is not explained. It appears that honeydew - feeding (i.e., feeding on a non - nectar sugar) is considered to be unimportant, yet, in the absence of nectar - producing plants, this may provide a reasonable explanation.

> "Non-nectar sugars, however, probably account for a very minor part of total carbohydrate intake, most coming from nectary sources. Shortage of nectar is probably not very often a serious nutritional problem for black flies. Conifers lack nectaries, but black flies are abundant (and clearly find plenty of sugar for flight energy) in coniferous forests of Canada and Siberia where there is generally less nectar than in most other vegetational zones."

A similar statement by Lewis and Domoney (1966) supports the theory that honeydew is an important sugar source for black flies, "In several of the drier parts of Africa the commonness of this liquid [i.e., crop liquid] was matched by the lack of any obvious source of it". This is essentially the exact observation of sand fly researchers in Africa who subsequently discovered aphid derived sugars, by High Performance Liquid Chromatography (HPLC) in samples of wild sand flies (Moore, *et al.*, 1987).

Sand Fly Studies

Three studies have been conducted on sand flies (Phlebotominae) to determine whether these flies use honeydew as a sugar source (Moore *et al.*, 1987; MacVicker *et al.*, 1990; Wallbanks *et al.*, 1990). In each of these studies, sugars such as melezitose (and the hydrolysis product turanose) and erlose (fructomaltose), which are considered to be specific to honeydew, were identified by HPLC within samples of flies (Appendix 5). In the studies by MacVicker *et al.* (1990) and Wallbanks *et al.* (1990), pooled samples of 20 - 30 sand flies were used in order to provide sufficient quantities of material (i.e., sugars) for analysis. Since the actual number of sand flies collected for analysis in both of these studies was low, few chromatographic analyses (< 10) were performed. HPLC analyses of individual flies were not attempted in these studies, possibly due to the small sample sizes in combination with insufficient concentrations of sugars per fly.

Lewis and Domoney (1966) estimated that the crop of *S. damnosum* can hold approximately 30 times more liquid than the crop of several species of sandfly (*Lutzomyia spp.*). In their study, Lewis and Domoney (1966) examined individual crop contents of each of these species using thin layer chromatography (T. L. C.) and were able to detect several sugars including: sucrose, glucose, fructose, maltose, melibiose and raffinose. Although sugars reported to be unique to

honeydew have not been detected in black flies, it is apparent that no attempt has been made to test sugars other than those likely to be found in nectar (Appendix 5).

It should be possible to detect sugars commonly found in honeydew such as melezitose and stachyose in individual black flies, in which case, the frequency of honeydew - feeding may be determined. In addition, it is possible to obtain sufficient numbers of specimens, in Algonquin Park, where black fly populations are very high.

Objectives

Alternative sources of sugar such as homopteran honeydew have generally been ignored by researchers. By employing T.L.C. techniques to identify sugars in individual black flies it should be possible to test the hypotheses that:

1) adult male and female black flies are not confined to ingesting flower nectar, and will readily consume non - floral sources of sugar, such as homopteran honeydew;

2) the type of sugar (i.e., nectar and / or honeydew) consumed by adult black flies will depend on the habitat in which they are found, and thus the availability of such sugar sources.

Similar studies have not been possible in sand flies, due to low numbers of flies collected and the small sugar meal sizes, requiring that samples be pooled. This study is possible in black flies, however, because population densities can be

very high in the study area, in Algonquin Park. Furthermore, the sugar meals taken by black flies are large enough to allow for T. L. C. testing of individuals.

Materials and Methods

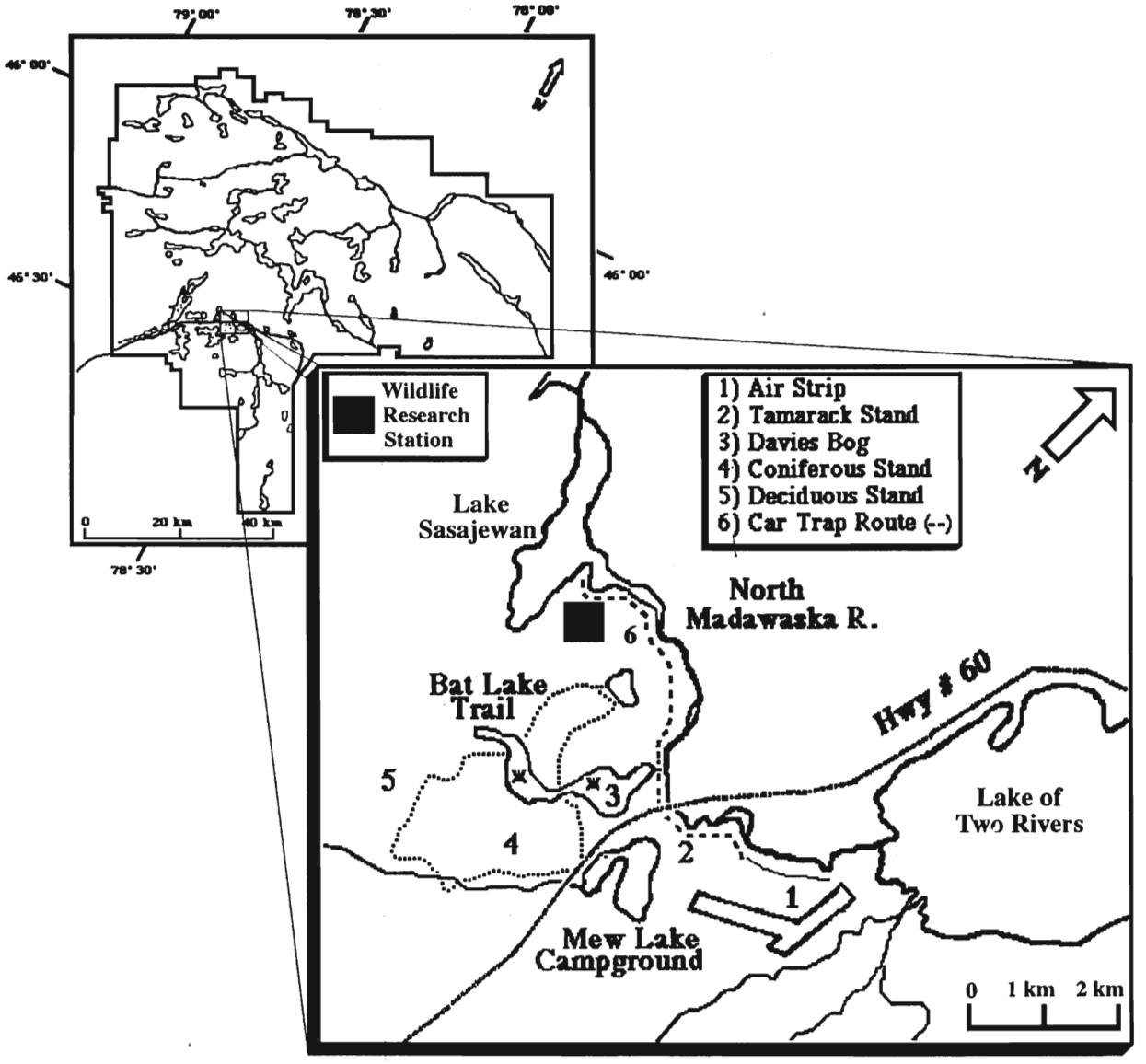
(A) Location

This study was conducted in Algonquin Provincial Park, Ontario, in the area of the Wildlife Research Station (45° 34' N, 78° 41' W) during the spring and summer of 1993 (Figure 1). Three collection strategies were used and are dealt with in separate sections: (1) Tamarack study, (2) Car trap samples and (3) Four habitat study. The tamarack site was chosen as these trees were found to be infested with honeydew - producing homopterans and therefore represented a distinct source of honeydew. Car trap samples were taken as it was known that large numbers of female and male black flies could be obtained in this manner. The four habitats were chosen as it was expected that sugar sources (both flower nectar and homopteran honeydew) available in each site may differ.

(1) Tamarack Study

A tamarack stand *Larix larcina* (Du Roi) Koch, infested with *Adelges lariciatus* (Patch) (Homoptera: Adelgidae), was located near the main office (collection site 2 in Figure 1) for the Mew Lake Campgrounds (at km 31 from West gate of Park). These homopterans were observed to be producing large droplets of honeydew from May 20 to June 25 after which point the amount of honeydew produced began to decrease. Six samples of honeydew were collected on each of June 02 and June 17 from homopterans infesting the tamarack stand.

Figure 1 Map of Algonquin Provinvial Park, Ontario and close up of the area in which this study was conducted.


79° 00'
78° 30'
78° 00'
46° 00'
46° 30'
46° 00'
78° 30'
0
20 km
40 km
Wildlife Research Station
1) Air Strip
2) Tamarack Stand
3) Davies Bog
4) Coniferous Stand
5) Deciduous Stand
6) Car Trap Route (--)
N
Lake Sasajewan
North Madawaska R.
6
Bat Lake Trail
Hwy # 60
5
3
4
2
1
Lake of Two Rivers
Mew Lake Campground
0 1 km 2 km

(2) Car Trap Study

Sampling was performed using a vehicle - mounted insect net (i.e., car trap). The opening dimensions of the net being $1m^2$. The route taken ran parallel to the North Madawaska River (collecting site 6 in Figure 1), passing close to a bog (Davies Bog), the homopteran infested tamarack stand and an open meadow (abandoned air field). Roadside vegetation consisted mostly of coniferous trees (*Pinus strobus* L., *Pinus resinosa* Ait. and *Picea glauca* (Moench) Voss).

(3) Four Habitat Study

An attempt was made to collect samples and identify the more common trees and flowering plants in each habitat used in this study (Appendix 6). Two open habitats (Davies Bog and air field sites) and two closed or forest habitats (deciduous and coniferous sites) were chosen. Bogs, clearings (due to fire or logging), deciduous and coniferous forests are all found within Algonquin Park and so the habitats were chosen to represent these areas.

Davies Bog

The collection site for Davies Bog was entered, from highway 60, approximately 0.5 km west of the entrance to the Mew Lake Campground. In order to gain access to Davies Bog from the highway at this point, it was necessary to walk (approximately 100 m) North through a boundary of pine trees (collection site 3 in Figure 1). From as early as May 08 the dominant flowering plant was *Chamaedaphne calyculata* (leather leaf). These were replaced over the course of the season by a variety of other Ericaceous shrubs excluding blueberries.

Abandoned Air Field

The air field is located approximately 0.5 km east of the main office of the Mew Lake Campground. Sweep samples were conducted approximately 100 m East of the parking lot, parallel to the North Madawaska River (collection site 1 in Figure 1). The air field is essentially an open meadow. The terrain is quite level, in the field, and the ground cover consists of reindeer moss (*Cladonia* sp.), grasses and low blueberry shrubs. Based on casual observations, the blueberries flowered from mid May to mid June. Samples of *Vaccinium angustifolium* nectar were taken on June 03 and June 17 from the air field.

Deciduous Dominated Habitat

The start of the deciduous site is located 1 km from the beginning of Bat Lake Trail (at km 30 from the West gate of the park). The stand of deciduous trees extends for approximately 500 m of the trail (collection site 5 in Figure 1). From the last week of May this habitat also appears quite closed-in as the trees and understory plants leaf out.

Coniferous Dominated Habitat

The coniferous site is located at the start of the Bat Lake Trail (at km 30 from the West gate of the park) and continues for the first 1km of the trail (collection site 4 in Figure 1). This sampling area is much more closed in than either the air strip or the Davies Bog sites due to the dense growth of coniferous trees which, in several sections, cover the area directly over the trail.

(B) Sampling Methods

(1) Tamarack Study

Standard insect nets (30 cm in diameter) were used during sweep collections of the tamarack stand. Sweep sampling was performed between 6:45 PM and 8:05 PM on the following nine dates: June 02, 12, 16, 17, 19, 24, 26, 28, and July 06. On each date, "early" and "late" sweeps were conducted (Appendix 7). The early sweep began immediately upon arrival at the tamarack stand. One investigator swept among the tamarack branches on one side of the stand, for 3 minutes, while a second swept the other side, for a total of 6 minutes of sweeping per sample. It was assumed that flies resting on the trees or feeding on the homopteran honeydew would be caught by this method. After a ten minute wait, the late sweep was conducted, with the two investigators repeating the sweeping procedure. It was assumed that flies caught during the late sample were residual on the tamarack (i.e., flies not caught during the early sweep) or were flies attracted to the area by the presence of the human collectors.

(2) Car Trap Study

Each car trap 'run' lasted 15 minutes and covered a distance of 5.8 km from the parking lot at the Wildlife Research Station cookhouse to the parking lot for the abandoned air field. On 8 sample days (May 21, May 27, June 03, June 10, June 17, June 24, July 02 and July 09), morning (10:00 - 11:45 AM) and evening (6:30 - 7:30 PM) car trap samples were obtained (Appendix 8). These times were chosen to coincide with known peaks in black fly activity, thus increasing the chances of finding recently sugar - fed black flies (Davies, 1952).

(3) Four Habitat Study:

Standard insect nets (30 cm in diameter) were used during 15 minute sweep collections through vegetation in the four sites. Morning (between 10:00 AM - 12:00 PM) and evening (between 6:00 - 8:05 PM) samples were obtained on 8 collection dates (May 21 and 27, June 03, 10, 16, and 24, July 02 and 09). The air field and coniferous sites were sampled simultaneously by two investigators, as were Davies Bog and the deciduous site, in both morning and evening samples (Appendices 9, 10, 11 and 12).

(C) Storage of Samples

(1) Tamarack Study

After each sweep collection the end of each insect net was sealed in a Ziploc® baggie and the nets were transported to the field laboratory where they were placed in a -20°C freezer to immobilize the flies. After approximately 15 minutes, the flies were removed from the insect nets, separated from any miscellaneous insects and plant debris, and placed in labeled 2 ml Nalgene® Cryovials. Flies were then frozen in liquid nitrogen and transported in liquid nitrogen to Brock University, where they were transferred to a -80°C freezer until used in T. L. C. analysis.

Collection and Storage of Honeydew

Samples of honeydew were collected by pressing No. 1 Whatman filter paper wicks to droplets directly associated with homopterans infesting the tamarack. The filter paper wicks were then placed into labeled 2 ml Nalgene® Cryovials and frozen in liquid nitrogen. Honeydew samples were transported in

liquid nitrogen to the University, where they were transferred to a -80°C freezer until used in T. L. C. analysis.

(2) Car Trap Study

After each collection the insect net was removed from the frame attached to the vehicle and placed in a -20°C freezer to immobilize the flies. After approximately twenty minutes, the contents of the car trap net were placed into one or two petri dishes and sorted under a dissecting microscope. Subsamples of 60 male and female black flies having noticeably distended abdomens were placed into labeled 2 ml Nalgene® Cryovials and were then frozen in liquid nitrogen. These subsamples remained in liquid nitrogen during transport to the University laboratory where they were then transferred to a -80°C freezer until used in T. L. C. analysis.

(3) Four Habitat Study

The black fly samples, in each habitat, were treated in the same way as the black fly samples from the tamarack site.

Collection and Storage of Nectar

On each date, four shrubs from different locations in the air field were sampled. Several flowers were probed in order to extract approximately 10 µl of nectar from a given shrub.

On June 03 disposable Drummond microcaps® (microcapillary tubes), with wire plunger, were used to probe the inside of *V. angustifolium* (blueberry) flowers. The nectar within each microcap was then placed onto No. 1 Whatman filter paper

squares. The filter paper squares were then placed into labeled 2 ml Nalgene® Cryovials and frozen in liquid nitrogen.

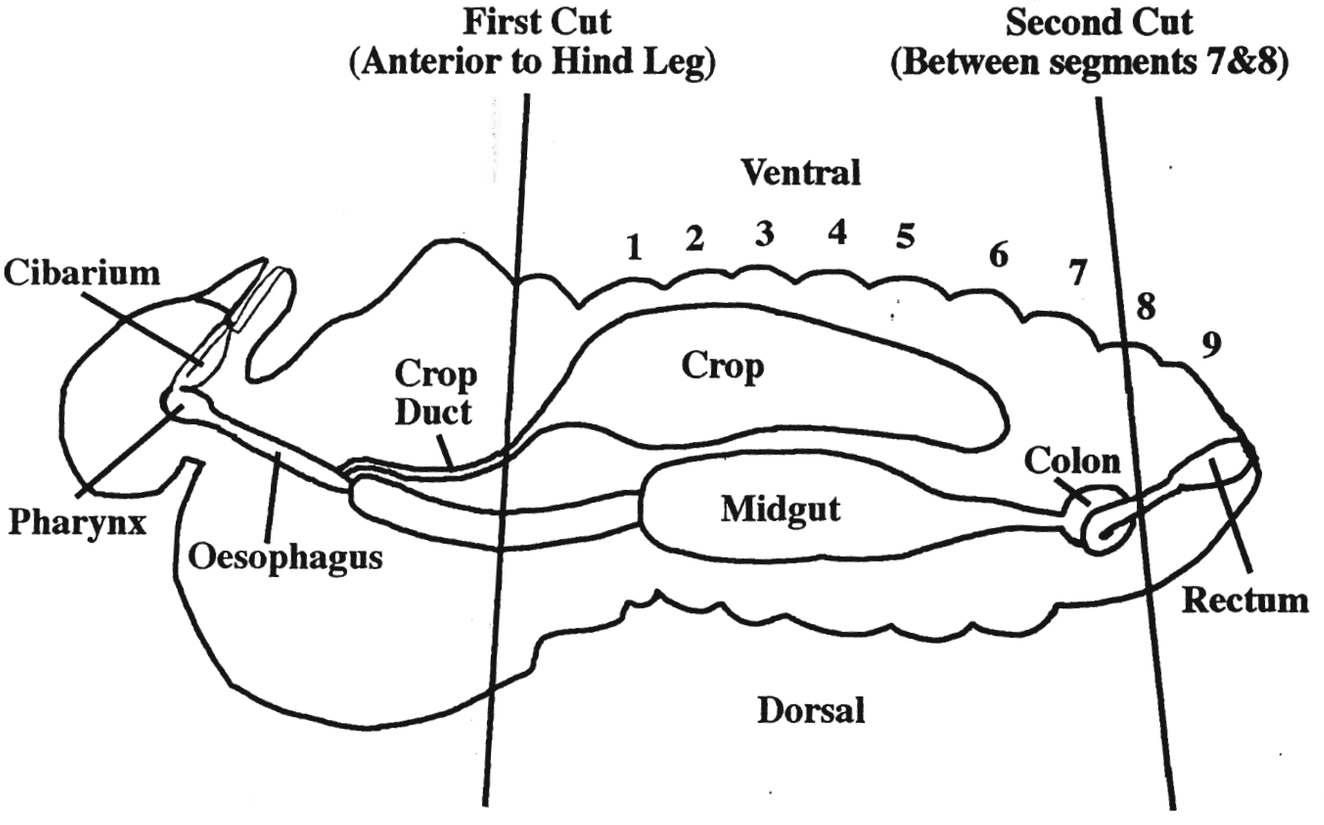
On June 17, No. 1 Whatman filter paper wicks were used to probe for nectar in the flowers of *V. angustifolium*. This method of collecting nectar was based on the methods of McKenna and Thomson (1988). The filter paper wicks were then placed into labeled 2 ml Nalgene® Cryovials and frozen in liquid nitrogen.

These samples remained in liquid nitrogen during transport to the University laboratory where they were then transferred to a -80°C freezer, at the University, until used in T. L. C. analysis.

(D) Dissection of Black Flies

Black flies were dissected on microscope slides placed under a dissecting microscope. Micro dissection scissors were used to cut through the black flies at a point just anterior to the hind legs. A second cut was then made to separate the genitalia from the abdomen (Figure 2). After dissection single black flies were placed in numbered wells of tissue culture plates. Each well contained approximately 2 ml of lactic acid which cleared the tissues providing an unobstructed view of taxonomically important features. Identification of black flies was aided by the taxonomic keys of Davies *et al.* (1962), and descriptions of Hunter (1990). After identification each individual could then be associated with the respective position of the gut contents on the T. L. C. plate. Although an attempt was made to differentiate between *S. venustum* and *S. rostratum*, it was sometimes difficult to do so. In such instances, flies were labeled as "*S. venustum / S. rostratum*".

Figure 2 Diagram showing the digestive tract of a black fly and the sections in which the black flies were cut during dissection.


First Cut
(Anterior to Hind Leg)
Second Cut
(Between segments 7&8)
Ventral
1
2
3
4
5
6
7
8
9
Cibarium
Crop
Duct
Crop
Colon
Midgut
Pharynx
Oesophagus
Rectum
Dorsal

(E) Thin Layer Chromatography Methods

T.L.C. was used as the method for separating sugars obtained from the crop and midgut of black flies. T. L. C. with cellulose as the layer has been used to separate and identify the sugars found in black flies (Lewis and Domoney, 1966). This method provided a very efficient method in terms of the information acquired, cost and time for studying the sugars from a large number of individual black flies.

T. L. C. Plates

T. L. C. plates were prepared using Sigmacell type 20 (cellulose powder), according to the manufacturer's instructions. A plate spreader was used to apply a 0.5 mm layer on the 20 x 20 cm glass plates.

Application of Standards and Samples to T. L. C. Plates

Standards

All test plates were 'spotted' at 28 points along the origin (2 cm from the base of the plate), at 0.6 cm intervals (leaving approximately 1.5 cm on each side of the plate). This was done to reduce the chances of identification error due to edge effects. Standard sugar solutions consisted of 0.1 g of each sugar dissolved in 10 ml of distilled water. Disposable Drummond microcaps® with wire plunger (hereafter termed microcaps) were used to dispense 1.5 µl of the standard sugar solution, containing approximately 15.0 µg of each sugar. Two standard solutions were used: Set 1 included fructose (Fru), glucose (Glc), sucrose (Suc), melezitose (Mez), raffinose (Raf) and stachyose (Sta); set 2 included galactose (Gal), turanose (Tur), melibiose (Mel), and maltose (Mal). Standard set 1 was applied to each plate, at points 1, 14 and 27, and set 2 was applied at points 2, 15, and 28. The

sugar trehalose which has been detected in the hemolymph of insects was used as a standard in preliminary trials only, as this sugar did not react with either detector spray.

Black Fly Samples

A microcap was used to place a 2.0 µl drop of distilled water onto a microscope slide adjacent to the dissected abdomen. This remaining portion of the abdomen, containing the crop and midgut, was macerated in the distilled water using fine probes (#0 insect pins). A microcap was then used to collect the liquid for application to a T. L. C. plate. Individual black fly gut contents were applied to each plate, at points 3 - 13 and 16 - 26.

Honeydew and Nectar Samples

A microcap was used to place a 2.0 µl drop of distilled water onto a microscope slide adjacent to filter paper squares which had been used to absorb honeydew or nectar, in the field. The filter paper was macerated in the distilled water using fine probes (#0 insect pins). A microcap was then used to collect the liquid for application to a T. L. C. plate.

Solvent

The solvent in which plates were run consisted of formic acid, methyl ethyl ketone, tertiary butanol and distilled water (15 ml: 25 ml: 35 ml: 25 ml) according to Damonte *et al.* (1971). Plates were placed into developing chambers in 100 ml of solvent for 2 hours. Fresh solvent was used for each run. This solvent was used to run all plates used in this study.

Developing Reagent (D. A. P. A.)

In preliminary trials, a detector spray modified from Damonte *et al.* (1971) and containing diphenylamine (2 g), aniline (2 ml), phosphoric acid (15 ml) and acetone (100 ml) was employed. After each plate was removed from the development tank and allowed to air dry, the D.A.P.A. reagent was sprayed onto the plate using a Crown® spray nozzle. The plate was then heated for 7-10 minutes using a hand held blow - drier. All sugars, except trehalose, undergo a colour reaction. With this spray, sugars appear in various colours thus providing further support for their identification (Figure 3). The reagent was stored at 4°C, and the remainder discarded after 3 days.

Developing Reagent (Urea)

The reagent used primarily in this study was modified from Bailey (1962), and consisted of urea (3 g), 1-butanol (90 ml) and phosphoric acid (25 ml). After air drying and being heated for 7-10 minutes, sugars containing a fructose (ketose) unit underwent a colour reaction, and were indicated by blue spots, which were readily distinguished from the white background. An advantage to this detector is that melibiose and maltose do not react, providing a more clear indication when melezitose is present (Figure 4). The sugars glucose and galactose also do not react with this reagent. The reagent was stored at 4°C, and the remainder discarded after 3 days.

It should be noted here that a dual spray system was attempted using the two reagents mentioned above. The urea reagent was sprayed onto a plate and developed. After recording the spots that appeared, the D. A. P. A. reagent was applied to the plate. It was thought that the sugars not detected by the first reagent

Figure 3 Photograph of T. L. C. plate developed using the D. A. P. A. reagent. The stachyose standard was not used in this plate although the hR_f value of the low migrating sugar in the two *S. venustum* lanes and the unidentified dipteran lane (from tamarack samples) correspond to the hR_f value of stachyose standards used in later trials. A sample of *Vaccinium angustifolium* (blueberry) nectar is also present on this plate. Standards include: sucrose (Suc), glucose (Glc), fructose (Fru), turanose (Tur), melezitose (Mez), maltose (Mal), raffinose (Raf), melibiose (Mel) and mannose (Man).

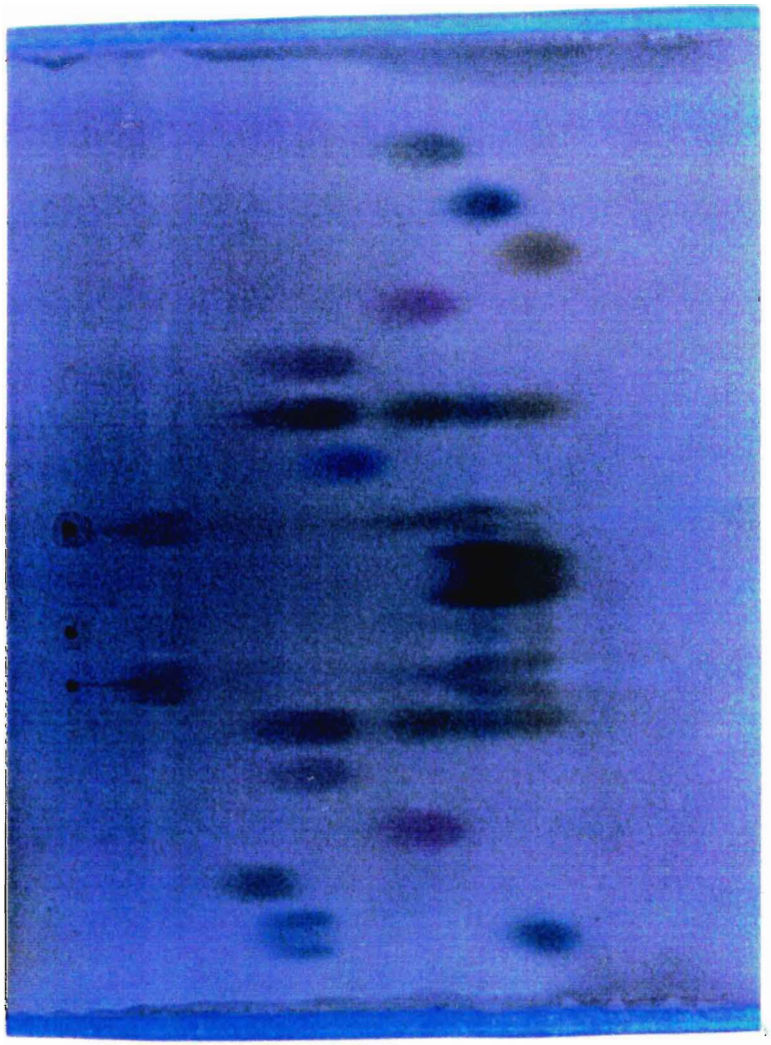
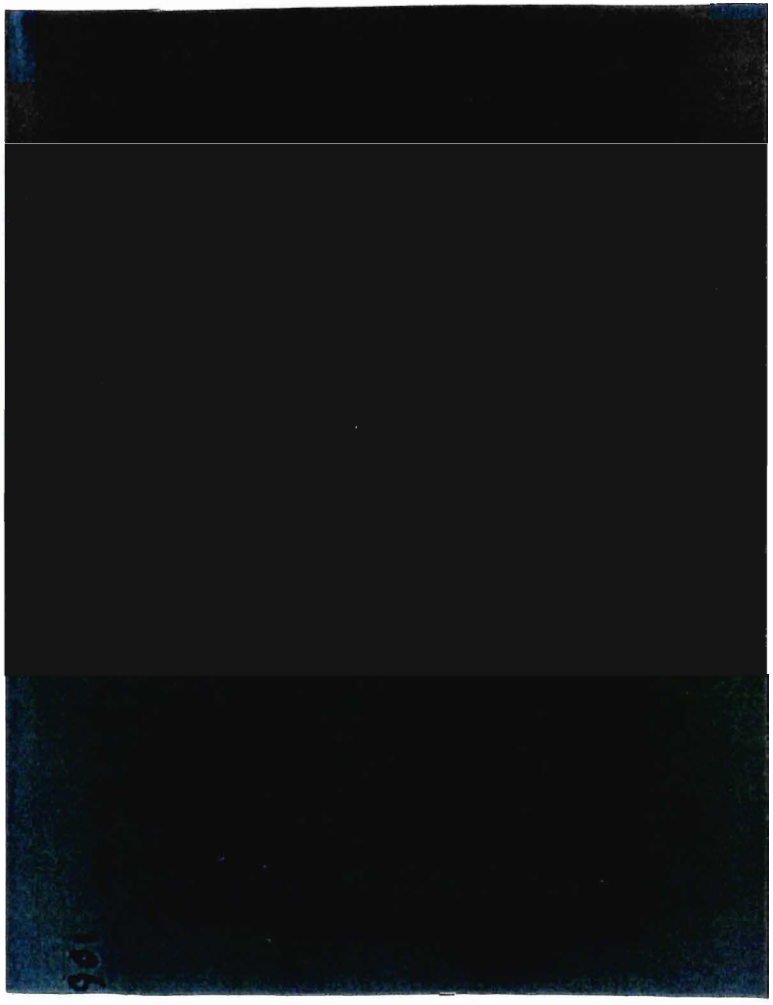

Suc
Glc
Fru
Tur
Mez
Mez / Suc / Glc / Fru
Mal
June 02 (5 female S. ven.)
V. angustifolium nectar
June 02 (3 male S. ven.)
June 02 (4 dipteran sp.)
Mez / Suc / Glc / Fru
Mez
Tur
Raf
Mel / Man

Figure 4 Photograph of T. L. C. plate developed using the Urea reagent. Lanes 1, 15,and 27 were spotted with standard (1) and lanes 2, 16 and 28 were spotted with standard (2). Standard (1) includes: fructose (Fru), glucose (Glc), sucrose (Suc), melezitose (Mez), raffinose (Raf) and stachyose (Sta). Standard (2) includes: galactose (Gal), turanose (Tur), melibiose (Mel) and maltose (Mal). The sugars Glc, Gal, Mel and Mal do not react with this reagent. Individual black fly gut contents were spotted on lanes 3 - 13 and 16 - 23.



Standard (1) 1
Standard (2) 2
Male *S. venustum* (June 12, early) 3
Male *S. venustum* (June 12, early) 4
Male *S. venustum* (June 12, early) 5
Male *S. venustum* (June 12, early) 6
Male *S. venustum* (June 12, early) 7
Male *S. venustum* (June 12, early) 8
Male *S. venustum* (June 12, early) 9
Male *S. venustum* (June 12, early) 10
Male *S. venustum* (June 12, early) 11
Male *S. venustum* (June 12, early) 12
Male *S. venustum* (June 12, early) 13
Male *S. quebecense* (June 12, early) 14
Standard (1) 15
Standard (2) 16
Female *S. venustum* (June 12, early) 17
Female *S. venustum* (June 12, early) 18
Female *S. venustum* (June 12, early) 19
Female *St. mulata* (June 12, early) 20
Female *S. venustum* (June 12, early) 21
Female *S. venustum* (June 12, late) 22
Female *S. venustum* (June 12, late) 23
Female *S. venustum* (June 12, late) 24
Female *S. venustum* (June 12, late) 25
Female *S. venustum* (June 12, late) 26
Standard (1) 27
Standard (2) 28

would react with the second spray, thus adding to the number of sugars that could be detected. This did not work, however, as the standard sugars (galactose, glucose, maltose and melibiose) failed to appear after the second development.

Numbers of Individuals Tested

(1) Tamarack Samples

The D. A. P. A. reagent was used to develop sugars from the gut contents of 20 *S. venustum / rostratum* from samples on June 02. The gut contents of these flies were pooled and were therefore not included in later analyses. The Urea reagent was used to develop sugars from the remaining 201 black flies captured from the tamarack stand. The gut contents of all 201 flies were tested individually on T. L. C. plates.

Six honeydew samples were run on T. L. C. plates and developed with both the D. A. P. A. and Urea reagents, for comparison with sugars found in black fly gut contents.

(2) Car Trap Samples

From each morning and evening car trap sample, on each of the 8 sample days, a subsample of 10 male and 10 female *S. venustum* was taken. In total, the gut contents of 320 black flies were run on T. L. C. and visualized using the urea reagent. In preliminary T. L. C. trials, 10 male and 10 female flies, from June 03 and 10 male and 10 female flies, from June 16 were run on T. L. C. plates and developed using the D. A. P. A. reagent. The gut contents of all black flies were tested individually on T.L.C. plates.

(3) Habitat Samples

For each habitat site sampled, a maximum of 22 female black flies were analysed by T.L.C. from each morning and evening sample, on each of the 8 sample days. In many samples fewer than 22 black flies were collected, in which case, all flies were examined. From the four areas sampled, a total of 809 black flies were analysed using T.L.C.: Davies Bog (n=307), air field (n=237), deciduous habitat (n=171) and coniferous habitat (n=94). The urea reagent was used to develop sugars for all 809 black flies. The gut contents of all black flies were tested individually on T.L.C. plates.

Nectar samples were run on T. L. C. plates and developed with both the D.A.P.A. and Urea reagents, for comparison with sugars found in black fly gut contents.

Recording and Preserving Information on T. L. C. Plates

After development, all spots, the origin and solvent front were traced onto drafting velum which was placed directly onto each plate. The hR_f values (hR_f = 100 x R_f) were then measured from these tracings.

$$R_f = \frac{\text{migration distance from origin to middle of sugar spot}}{\text{migration distance from origin to solute front}}$$

After the plates were traced, they were stored in a refrigerator at 4°C, to preserve the colours, until a sufficient number were available to be photographed. Photographs were taken of the selected plates, under natural light conditions using Kodak Gold colour film. The hR_f values for the standards, honeydew, *V. angustifolium* (blueberry) nectar and black fly gut content sugars were calculated.

Sugar Combinations and Profiles

By using the urea reagent, 4 sugars were identified with some certainty, including: (1) fructose (the glucose component was assumed to be present, based on preliminary tests using the D. A. P. A. reagent), (2) melezitose (melibiose and maltose also have similar hR_f values but fortunately are not detected with the urea reagent), (3) raffinose and (4) stachyose. Similar migration distances prevent reliable discrimination between sucrose and turanose and for this reason sugars migrating to the level of these sugars were designated sucrose / turanose.

The fact that these five sugars could occur in a variety of combinations was considered. In order to determine the frequency of any particular combination of sugars, the data for individual black flies were recorded. From these data, the frequency of each sugar occurring in black flies from different habitats was also determined.

Within this study, the sugars melezitose and stachyose are of particular importance, as these sugars are known to occur in the honeydew of several homopterans from a variety of plants. For this reason, the sugar combinations (of the five sugars) were grouped into 5 sugar profiles. The 5 profiles are as follows: **(A)** Fru and Glc only, **(B)** combinations including Mez but excluding stachyose, **(C)** combinations including both Sta and Mez, **(D)** combinations including Sta, but excluding Mez and **(E)** combinations including Suc / Tur and / or Raf but excluding sugars other than Fru, Glc.

Statistical Analyses

The Cochran correction factor was used in all Chi - square tests, as this provides a good general method for reducing the risk of Type 2 error, especially when row and / or column totals of contingency tables are not set prior to experimentation (Zar, 1984).

From the tamarack study, a Chi - square test was used to determine whether the total number of male and female black flies captured differed significantly.

From the car trap study, a Chi - square test was used to determine whether the total number of male and female black flies captured differed significantly.

Only the sugar combinations determined from black flies tested individually on T. L. C. plates and developed using the urea reagent were used in Chi - square tests. Black flies containing no sugars were not included in the Chi - square analyses.

From the tamarack study, Chi - square tests were used to determine whether the presence and absence of the adelgid honeydew sugar (stachyose) differed significantly between male and female flies from early and late sweep samples.

From the car trap study, Chi - square tests were used to determine whether the presence and absence of the honeydew sugars (stachyose and melezitose) differed significantly between male and female flies from AM and PM sweep samples.

From the four habitat study, Chi - square tests were used to determine whether the presence and absence of the honeydew sugars (stachyose and melezitose) differed significantly between habitats for *S. venustum* as well as for the other species found within each habitat.

Seasonal Trends

It was thought that the number of sugar sources may tend to increase as the season progresses from mid - late spring to early summer. Therefore, the correlation between the time of year and the number of sugar combinations present in the guts of black flies from car trap samples and the four habitat study was investigated. The r^2 values for each site were determined.

(F) Behavioural Observations

The tips of tamarack branches infested with *Adelges lariciatus* (Homoptera: Adelgidae) were transported in Ziploc® baggies to the field laboratory at the Wildlife Research Station. Small branches were then placed into a petri dish and the dish positioned under a JVC® video camera, equipped with a Sigma® macro lens. Adult male and female *Simulium venustum* Say, reared from pupae by the method of Hunter *et al.* (1994), were introduced into the petri dish and the lid closed. Observations were recorded on videotape.

Results

(A) Numbers of Black Flies

There were significantly more male flies in the coniferous habitat than in the Davies Bog, air field and deciduous habitats (X^2 = 13.9, df=1, $P < 0.001$). The number of male flies collected from the tamarack stand, however, was significantly greater than the number in the coniferous habitat. (X^2 = 20.6, df=1, $P < 0.001$) (Table 1).

(1) Tamarack Study

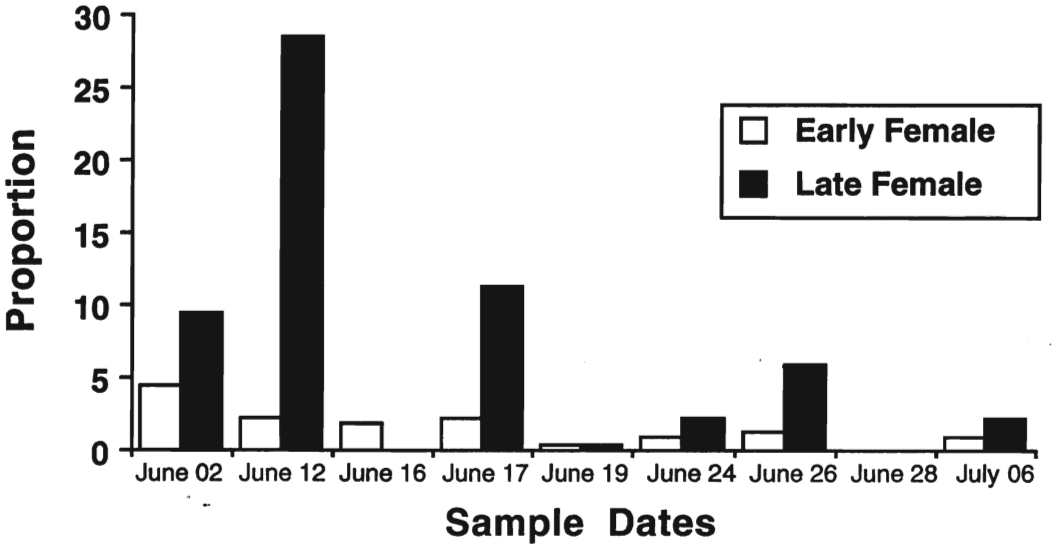
A total of 221 (165 female, 56 male) black flies (Appendix 7), representing six species, were captured from the tamarack stand, including: *S. venustum* Say (138 female, 51 male), *S. rostratum* Lundstroem (13 female, 1 male), *S vittatum* (Zetterstedt) (6 female, 2 male), *Stegopterna mutata* (Malloch) (6 female, 0 male), *S. aureum* Fries (2 female, 1 male), and 1 male *S. quebecense* Twinn (Appendix 8). All six species were present in early samples and all but *S. quebecense* were represented in the late samples. *S. venustum* comprised 83.3 % and 86.5 % of individuals in the early and late samples, respectively. The frequencies of female and male flies differed significantly between early and late sweep samples (X^2 = 33.0, df =1, $P < 0.001$). On six of the sample days (June 02, 12, 17, 24, 26, and July 06), the number of female black flies was greater in late net sweeps than in early sweeps (Figure 5a). An opposite trend was observed in male black flies (Figure 5b) as numbers were lower in late net sweeps than in early sweeps on 7 sample days (June 02, 12, 19, 24, 26, 28, and July 06).

All black flies collected from the tamarack stand were dissected for T. L. C. analysis. In sections B and C, results are presented with respect to the sugars found within each individual.

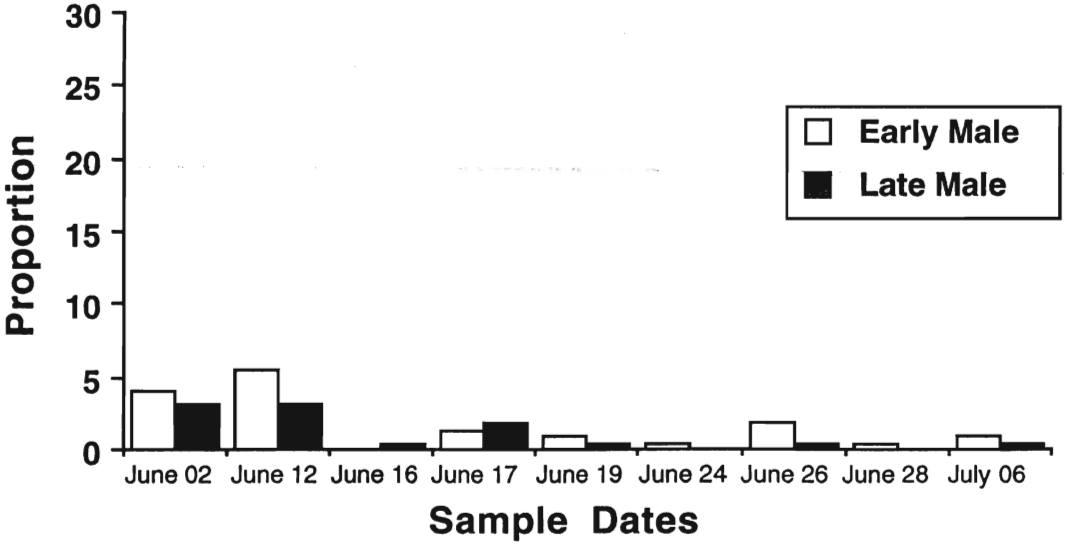
Table 1 The number (percent) of male flies collected from tamarack, car trap, Davies Bog, air field, deciduous habitat and coniferous habitat.

Location	Number (Percent) Males		Total No. Flies
Car trap Samples	5002	(65.39%)	7649
Tamarack Stand	56	(25.34%)	221
Davies Bog	1	(0.08%)	1216
Air Field	2	(0.05%)	398
Deciduous Habitat	1	(0.04%)	220
Coniferous Habitat	3	(3.09%)	97

Figure 5 Proportions from the total number of (a) female and (b) male black flies caught during early and late insect net sweep samples of tamarack on 9 sample days.



(5a) Proportions from the total number of flies collected from tamarack samples. (Female)



(5b) Proportions from the total number of flies collected from tamarack samples. (Male)

(2) Car Trap Study

A total of 7649 black flies were collected from AM and PM car trap samples on 8 sample days (Appendix 9). Significantly more males (5002 individuals) than females (2647 individuals) were collected in AM and PM car trap samples (X^2 = 20.86, df = 1, $P < 0.001$). The largest number of male and female flies collected from the car trap was on June 03 (Appendix 9). The number of female flies declines from June 03 to June 16 and increases again on June 24 (Figure 6 a). The number of male flies from May 27, June 10, June 16, and June 24 is relatively constant. However, a large peak in the number of male flies was found on June 03 (Figure 6 b). The number of flies was largest in PM than AM samples in 6 of the 8 sample days for both males and females (Figure 6 a, b).

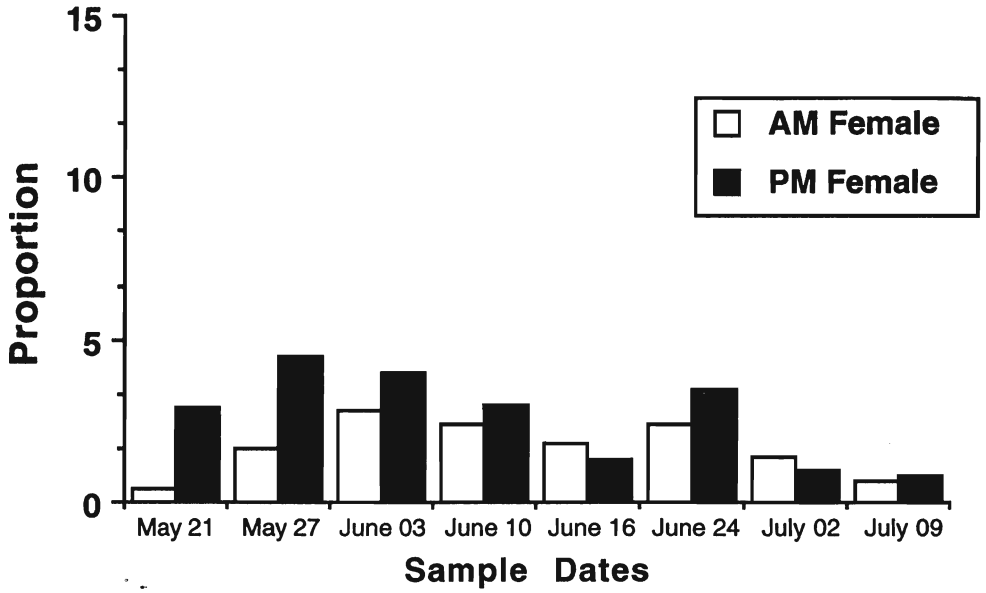
Only 20 flies (10 female and 10 male) were selected for sugar analysis, from both AM and PM samples on the 8 sample days. Of all flies collected, therefore, 320 (4.2%) were dissected for T. L. C. analysis and identification (Appendix 9). An attempt was made to select only *S. venustum* flies for analysis, but it is possible that a few *S. rostratum* were also included.

(3) Four Habitat Study

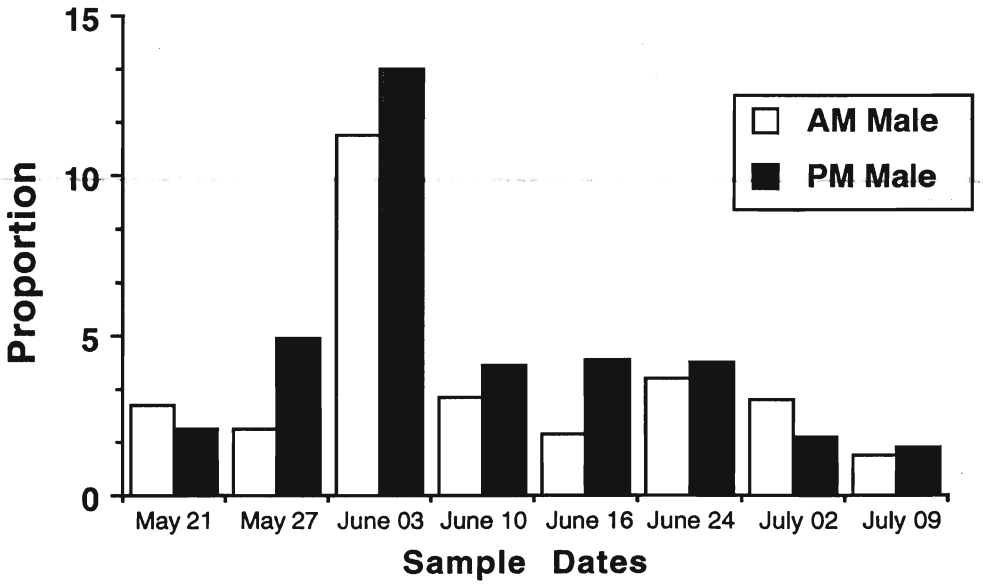
Davies Bog

A total of 1216 (1215 female, 1 male) black flies were collected from Davies Bog. From the 8 sample days, 457 flies were collected during AM samples and 759 were collected in PM samples (Appendix 10). The 9 species identified from Davies Bog include: *S. venustum*, *S. rostratum*, *S. tuberosum*, *S. aureum*, *S. vernum*, *S. parnasum*, *S. euryadminiculum*, *S. vittatum*, and *P. fuscum / mixtum* (Appendices 11 and 12). The number of female flies captured from Davies Bog is highest on May 27, June 10 and June 16 (Appendix 10). The number is much

Figure 6 Proportions from the total number of (a) female and (b) male black flies collected from AM and PM car trap samples.



(6a) **Proportions from the total number of flies collected from car trap samples. (Female)**



(6b) **Proportions from the total number of flies collected from car trap samples. (Male)**

lower on June 03. The number of flies was higher in PM than AM samples in 5 of the 8 sample days (Figure 7).

Of the 1216 black flies collected, 307 (25.2%) of the flies (all female) were dissected for T. L. C. analysis and identification (Appendix 10). Of these, 54.7% were *S. venustum*. *S. euryadminiculum*, *S. rostratum* and *S. vittatum* accounted for 15.0%, 13.4% and 11.1%, respectively (Appendix 11).

Air Field

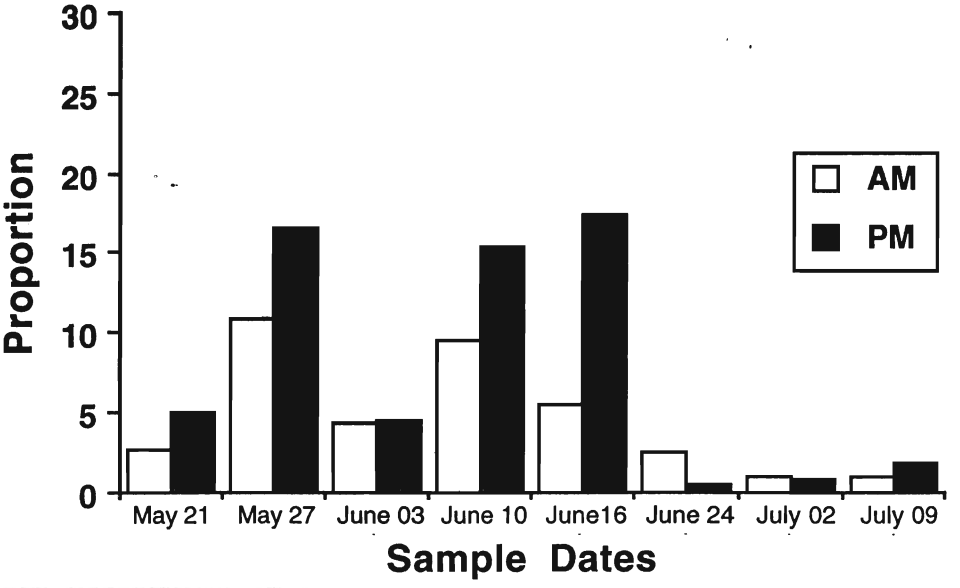
A total of 398 (396 female, 2 male) black flies were collected from the air field. From the 8 sample days, 159 flies were collected during AM samples and 239 were collected in PM samples (Appendix 13). The 10 species identified from the air field include: *S. venustum*, *S. rostratum*, *S. tuberosum*, *S. vittatum*, *S. euryadminiculum*, *S. aureum*, *S. decorum*, *S. parnasum*, *S. quebecense* and *P. fuscum / mixtum* (Appendices 11 and 14). The number of female flies captured from the air field is highest on May 27. The number is much lower on June 03 and increases again until June 16 after which time the number decreases. The number of flies was higher in PM than AM samples on 4 of the 8 sample days (Figure 8).

Of the 398 flies collected, 237 (59.6%) (all female) were dissected for T. L. C. analysis and identification (Appendix 13). *S. venustum* accounted for 63.3% of the individuals analysed from this site. The next most abundant species was *S. rostratum* (18.6%) (Appendix 11).

Deciduous Habitat

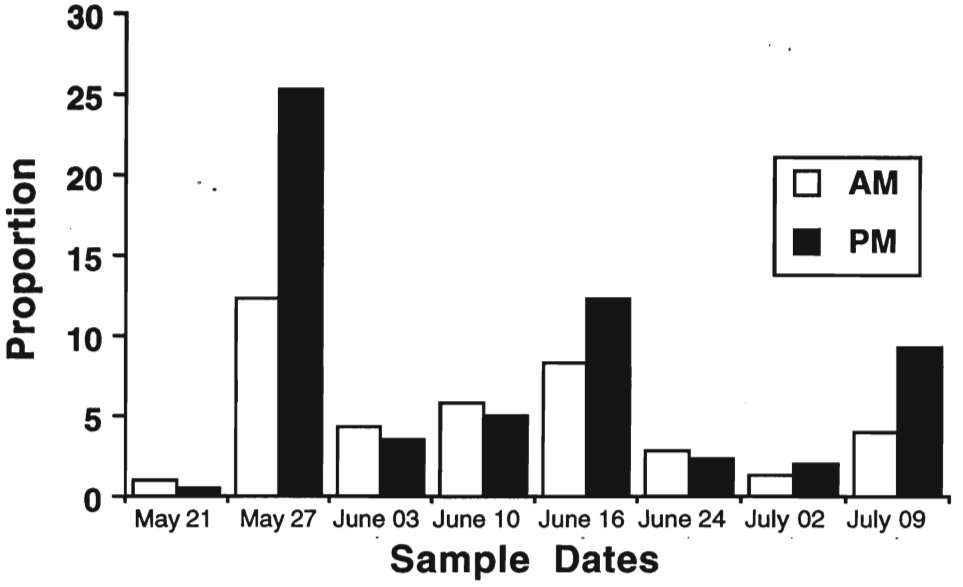
A total of 220 (219 female, 1 male) black flies were collected from the deciduous habitat. From the 8 sample days, 107 flies were collected during AM samples and 113 were collected in PM samples (Appendix 15). The 11 species

Figure 7 Proportions from the total number of black flies collected from Davies Bog on 8 sample dates.



(7) Proportions from the total number of flies collected from Davies Bog samples.

Figure 8 Proportions from the total number of black flies collected from the Air Field on 8 sample dates.



(8) Proportions from the total number of flies collected from air field samples.

identified from the deciduous habitat are as follows: *St. mutata*, *S. venustum*, *P. fontanum*, *S. rostratum*, and *P. fuscum / mixtum*, *S. vittatum*, *S. tuberosum*, *S. vernum*, *S. quebecense*, *S. decorum* and *S. aureum* (Appendices 11 and 16). The most flies were collected on May 27 and June 03. No flies were present in the AM and PM samples from June 16. The number of flies was greater in PM than AM samples on 3 of the 8 sample days (Figure 9).

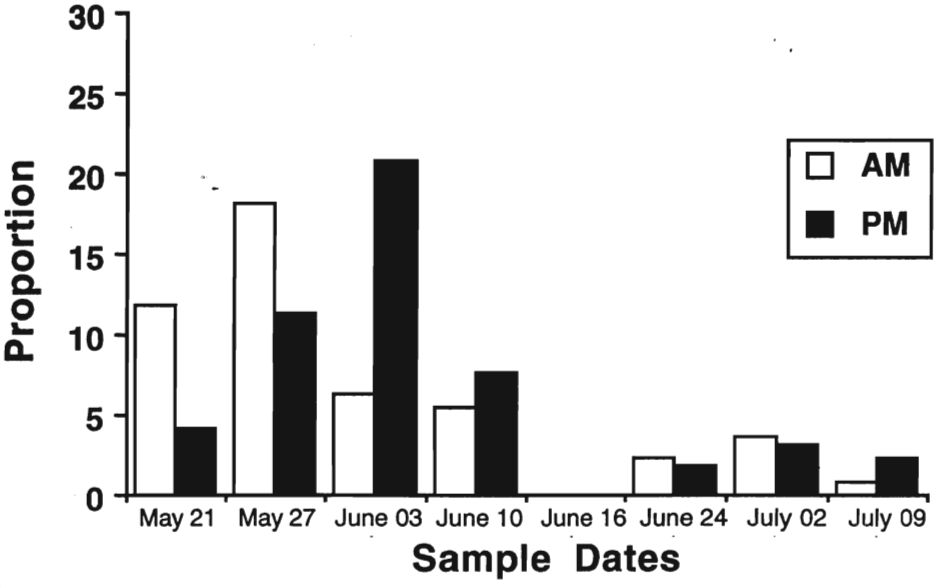
Of the 220 flies collected, 171 (77.7%) (all female) were dissected for T. L. C. analysis and identification (Appendix 11). *S. venustum*, *St. mutata*, and *P. fontanum* accounted for 34.5%, 36.8% and 14.6%, respectively, of the individuals analysed from this site (Appendix 15).

Coniferous Habitat

A total of 97 (94 female, 3 male) black flies were collected from the coniferous habitat (Appendix 17). From the 8 sample days, 54 flies were collected during AM samples and 43 were collected in PM samples. The 12 species identified from the coniferous habitat are as follows: *S. venustum*, *S. quebecense*, *S. vernum*, *S. euryadminiculum*, *S. tuberosum*, *S. rostratum*, *St. mutata*, *S. aureum*, *Ectemnia invenusta*, *S. croxtoni*, *S. parnasum* and *S. decorum* (Appendices 11 and 18). *S. parnasum* was absent from AM samples and *S. aureum*, *S. croxtoni* and *S. decorum* were absent from PM samples. The largest numbers of flies were collected on May 27 and June 03. No flies were present in the AM and PM samples from June 16. The number of flies was larger in PM than AM samples on 1 of the 8 sample days (Figure 10).

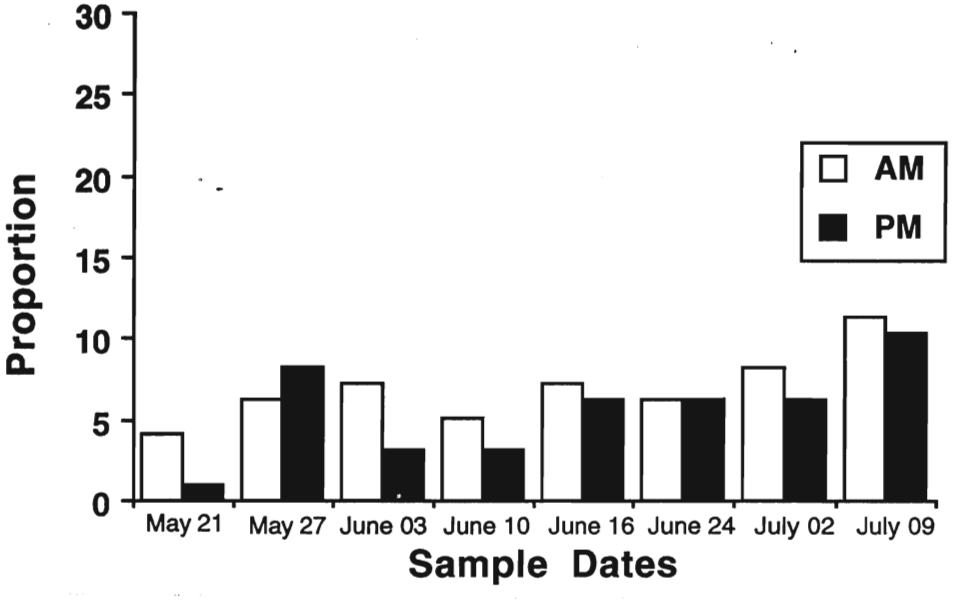
Of the 97 flies collected, 94 (96.9%) (all female) were dissected for T. L. C. analysis and identification (Appendix 17). The species *S. venustum* and *S. quebecense* accounted for 35.1% and 22.3%, respectively, of the individuals collected from this site (Appendix 11).

Figure 9 Proportions from the total number of black flies collected from the deciduous habitat on 8 sample dates.



(9) Proportions from the total number of flies collected from deciduous habitat samples.

Figure 10 Proportions from the total number of black flies collected from the coniferous habitat on 8 sample dates.



(10) Proportions from the total number of flies collected from coniferous habitat samples.

(B) Thin Layer Chromatography Analyses

hR_f Values (Standards)

Based on the hR_f values it is apparent that the migration distances for the sugars sucrose and turanose broadly overlap (Appendix 19). The sugars melezitose and maltose as well as melezitose and melibiose also migrate similar distances with the T. L. C. system employed in this study (Appendix 19).

hR_f Values (Honeydew)

Honeydew sample hR_f values from June 02 and 17 are given in Appendix 20. Five sugars were found within the samples of honeydew, including fructose, glucose, sucrose / turanose, raffinose and stachyose. Fructose and glucose occur in all samples developed by the D.A.P.A reagent, except in sample 3, from June 02, where both are absent. It is possible that glucose is also present in samples developed using the urea reagent; however, this reagent does not react with glucose. Stachyose was present in all but sample 1 from June 17 and was the only sugar present in sample 3. Although the sugar melezitose was also used as a standard, this sugar was not present in any of the honeydew samples from the homopterans infesting the tamarack. The sugars galactose, maltose, and melibiose also appeared to be absent.

hR_f Values (Nectar)

V. angustifolium nectar sugar hR_f values, from June 03 and 17 are given in Appendix 21. Samples developed using the D. A. P. A. reagent all contained the sugars fructose and glucose. The sugar maltose was present in 4 of the 7 samples.

The sugar migrating to the level of sucrose / turanose was present in 3 of the 7 samples. Samples developed using the urea reagent are missing the glucose and maltose spots, as these sugars do not react with the urea reagent.

hR_f Values (Black Flies)

The values of sugars from individual black flies developed using the D. A. P. A. and urea reagents are presented in Appendices 22 and 23, respectively. Nine sugars were identified in sugars from 40 black flies developed with the D. A. P. A. reagent, in preliminary trials. Fructose and glucose were present in all individuals.

(1) Tamarack Study

The sugar combinations of 194 black flies from early and late tamarack sweeps are presented in Appendices 24 and 25, respectively. The sugar combinations of 3 male and 5 female *S. venustum* from early sweeps on June 02 were not included as these were pooled samples. Similarly, the combinations of 4 male and 8 female *S. venustum* from late sweeps on June 02 were not included. From the 201 flies tested, 194 flies were found to contain sugars (no sugars could be detected in 6 *S. venustum* and 1 *S. aureum*). These sugars were found in 15 combinations: **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, and **(16)** Raf, Sta (Appendices 24 and 25). The numbers and percentages of *S. venustum* and species other than *S. venustum* containing sugars conforming to each combination were determined (Appendix 26). Combinations 4, 9 and 14 were found in *S.*

venustum but did not occur in any of the 5 other black fly species collected from the tamarack stand. In *S. venustum*, combination 1 (Fru, Glc only) occurred most often (53 individuals). Individual sugars occurred in *S. venustum*, with the following frequencies: Fru, Glc 96.9%, Suc/Tur 36.2%, Mez 7.4%, Raf 18.4% and Sta 49.7%. Individual sugars occurred in species other than *S. venustum*, with the following frequencies: Fru, Glc 96.8%, Suc/Tur 48.4%, Mez 29.0%, Raf 12.9% and Sta 25.8%.

(2) Car Trap Study

As only flies with distended abdomens were selected for analysis, all 320 flies were found to contain sugars. These sugars were found in 14 different combinations (see combination numbers 1 - 14 in tamarack study, above; Appendix 27). Sugar combinations **(15)** Sta, and **(16)** Raf, Sta were not found in flies from car trap samples. The numbers and percentages of male and female *S. venustum* containing sugars conforming to each combination were determined (Appendix 28). Individual sugars occurred with the following frequencies: for females, Fru, Glc 100.0%, Suc/Tur 59.4%, Mez 34.4%, Raf 22.5% and Sta 11.9%, and males Fru, Glc 100.0%, Suc/Tur 41.3%, Mez 26.3%, Raf 15.0% and Sta 5.6%.

(3) Four Habitat Study

The sugar combinations **(15)** Sta, and **(16)** Raf, Sta were not found in flies from any of the four habitat sites. The 14 sugar combinations (see combination numbers 1 - 14 in tamarack study, above) were found within flies from Davies Bog. Black flies from both the air field and the deciduous habitat contained only 12 of the 14 sugar combinations. Flies from the coniferous habitat contained 9 of the 14 sugar combinations.

Davies Bog

Of 307 female black flies tested by T. L. C. sugars were detected in 294 (95.8%) of the flies. No sugars were detected in 7 *S. venustum* and 1 *S. euryadminiculum* from AM samples (Appendix 29), and 4 *S. venustum* and 1 *S. euryadminiculum* from PM samples (Appendix 30). The sugars were found in 14 different combinations for *S. venustum* and 12 combinations for all species other than *S. venustum* (combinations 9, and 12 were not found). The three most frequently occurring combinations for *S. venustum* were 1, 2 and 5 and for species other than *S. venustum* 1, 2 and 6 (Appendix 31).

Individual sugars occurred with the following frequencies: for *S. venustum* Fru, Glc 100.0%, Suc/Tur 34.6%, Mez 16.7%, Raf 12.8% and Sta 19.9%; and for species other than *S. venustum* Fru, Glc 100.0%, Suc/Tur 55.8%, Mez 24.6%, Raf 15.2% and Sta 12.3%.

Air Field

Of 237 female black flies tested by T. L. C. sugars were detected in 228 (96.2%) of the flies. No sugars were detected in 2 *S. venustum* and 1 *S. decorum* from AM samples (Appendix 32), and 4 *S. venustum* and 2 *S. rostratum* from PM samples (Appendix 33). The sugars were found in 10 different combinations for *S. venustum* (combinations 4, 7, 9 and 12 were not found) and 9 combinations for all species other than *S. venustum* (combinations 3, 5, 8, 9 and 12 were not found). The three most frequently occurring combinations for *S. venustum* were 1, 2 and 6 and for species other than *S. venustum* were 1, 2 and 6 (combinations 6 and 13 occurred in 6 individuals each) (Appendix 34).

Individual sugars occurred with the following frequencies: for *S. venustum* Fru, Glc 100.0%, Suc/Tur 30.6%, Mez 16.0%, Raf 6.3% and Sta 7.6%; and for species other than *S. venustum* Fru, Glc 100.0%, Suc/Tur 53.6%, Mez 17.9%, Raf 17.9% and Sta 16.7%.

Deciduous Habitat

Of 171 female black flies tested by T. L. C. sugars were detected in 160 (93.6%) of the flies. No sugars were detected in 4 *St. mutata*, 1 *S. venustum*, 1 *S. aureum* and 1 *S. vernum* from AM samples (Appendix 35), and 3 *St. mutata* and 1 *P. fontanum* from PM samples (Appendix 36). The sugars were found in 11 different combinations for *S. venustum* (combinations 8, 9 and 12 were not found) and 9 combinations for all species other than *S. venustum* (combinations 4, 5, 9, 12, and 14 were not found). The three most frequently occurring combinations for *S. venustum* were 1, 2 and 6, and for species other than *S. venustum* 1, 2 and 6 (Appendix 37).

Individual sugars occurred with the following frequencies: for *S. venustum* Fru, Glc 100.0%, Suc/Tur 33.5%, Mez 31.0%, Raf 12.1% and Sta 10.3%; and for species other than *S. venustum* Fru, Glc 100.0%, Suc/Tur 45.1%, Mez 29.4%, Raf 3.9% and Sta 9.8%.

Coniferous Habitat

Of 94 female black flies tested by T. L. C. sugars were detected in 91 (96.8%) of the flies. No sugars were detected in 1 *S. venustum*, 1 *S. quebecense* and 1 *S. tuberosum* from AM samples (Appendix 38), whereas sugars were detected in all flies from PM samples (Appendix 39). The sugars were found in 6 different combinations for *S. venustum* (combinations 4, 5, 7, 8, 11, 12, 13 and 14 were not found) and 8 combinations for all species other than *S. venustum* (combinations 4, 5, 9, 11, 12 and 14 were not found). The three most frequently

occurring combinations for *S. venustum* were 1, 2 and 6 (sugar combinations 6 and 10 were found in 3 individuals each) and for species other than *S. venustum* 1, 2 and 6 (Appendix 40).

Individual sugars occurred with the following frequencies: for *S. venustum* Fru, Glc 100.0%, Suc / Tur 34.4%, Mez 21.9%, Raf 12.5% and Sta 3.1%; and for species other than *S. venustum* Fru, Glc 100.0%, Suc/Tur 55.9%, Mez 28.8%, Raf 17.0% and Sta 5.1%.

Sugar Profiles

The sugar combinations were grouped into five profiles: **(A)** Fru and Glc only (i.e., combination 1), **(B)** combinations including Mez (i.e., combinations 3, 6, and 10), **(C)** combinations including both Sta and Mez (i.e., combinations 11, 12, and 14), **(D)** combinations including Sta, but excluding Mez (i.e., combinations 5, 8, 9, 13, 15, and 16) and **(E)** combinations including Suc/Tur and / or Raf but excluding sugars other than Fru, Glc (i.e., combinations 2, 4, and 7). The number and proportion of individuals tested by T. L. C. in the Tamarack, car trap, and four habitat studies are given in Table 2. Of all 1287 flies tested in this study 441 (34.3%) contained melezitose and / or stachyose sugars.

(1) Tamarack Study

The male and female black flies from early and late collections corresponding to these five profiles were enumerated (Table 3). The proportion of flies corresponding to profiles A, B, C, D and E are, 32.0%, 7.2%, 3.6%, 42.3% and 15.0%, respectively. For female flies, profiles B and C account for 11.1% of the sugars detected from early samples and 14.9% from late samples (Table 3; Figure 11 a). No male flies contained the sugar melezitose which is within both profiles B and C (Table 3; Figure 11 b). For male flies profile D accounts for 71.4% of the sugars detected from early samples and 88.9% from late samples. For female flies, profile D accounts for 44.4% of the sugars

Table 2 Number (percent) of black flies containing various sugar profiles from tamarack, car trap, Davies Bog, air field, deciduous and coniferous habitats.

Location	Sugar Profiles* A		B, C and D		E		Total
Tamarack	62	(32.0)	104	(53.6)	28	(14.4)	194
Car Trap	143	(44.7)	108	(33.8)	69	(21.6)	320
Davies Bog	105	(35.7)	100	(34.0)	89	(30.3)	294
Air Field	134	(58.8)	48	(21.1)	46	(20.2)	228
Deciduous	75	(46.9)	53	(33.1)	32	(20.0)	160
Coniferous	43	(47.3)	28	(30.8)	20	(22.0)	91
Total	562	(43.7)	441	(34.3)	284	(22.0)	1287

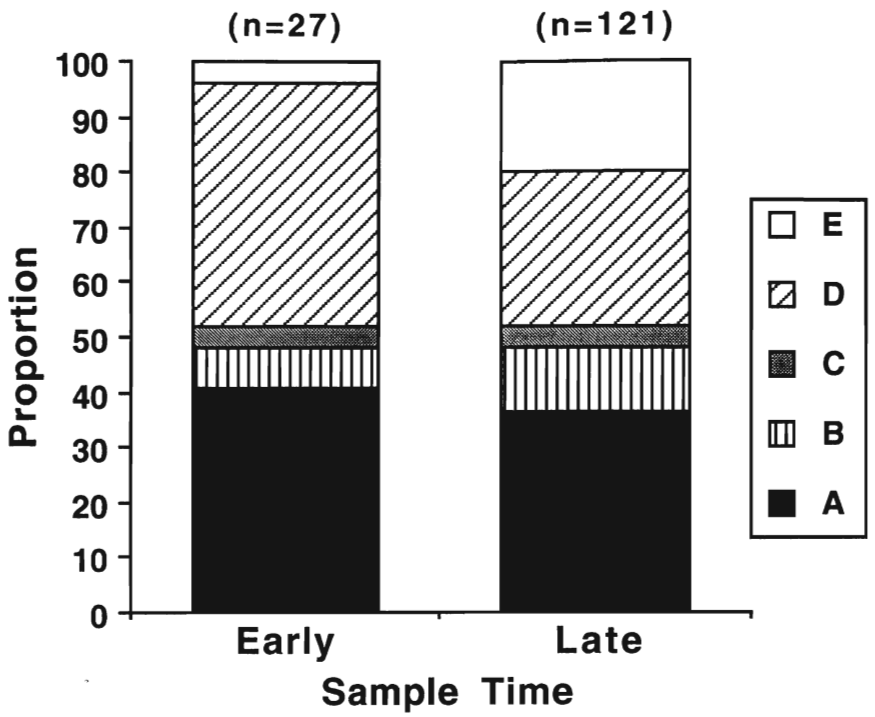
* A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations), D (Sta combinations) and E (Suc / Tur, and / or Raf combinations).

Table 3 Total number of male and female black flies containing sugar combinations conforming to each of 5 profiles from early and late sweep collections of tamarack.

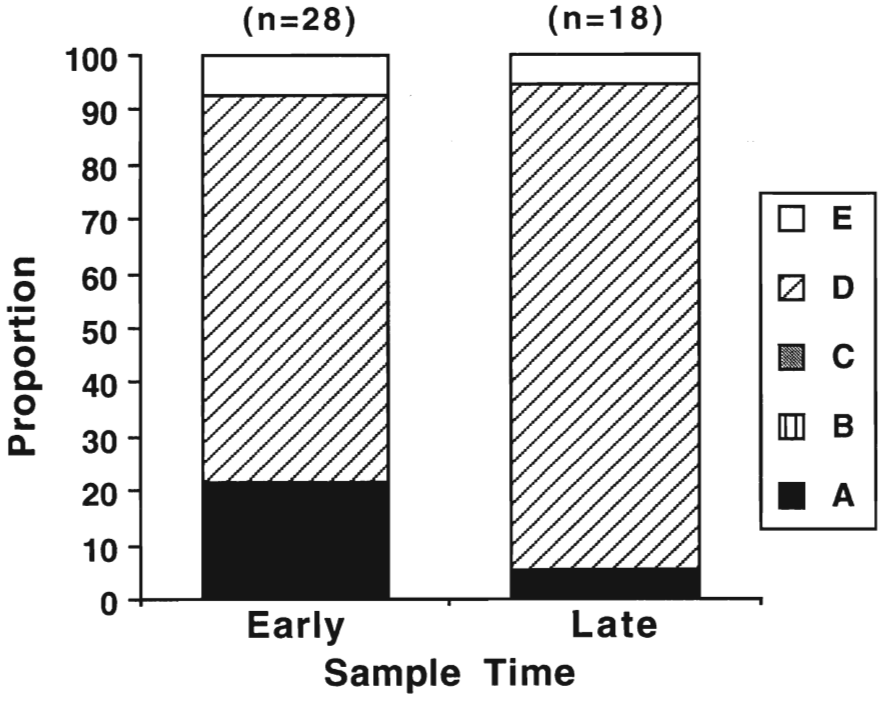
Time / Sex	Sugar Profiles* A	B	C	D	E	Total
Early Female	11	1	2	12	1	27
Early Male	6	0	0	20	2	28
Early Total	17	1	2	32	3	55
Late Female	44	13	5	34	25	121
Late Male	1	0	0	16	1	18
Late Total	45	13	5	50	26	139
Overall Total	62	14	7	82	29	194

* A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations), D (Sta combinations) and E (Suc / Tur, and / or Raf combinations).
Note: This table does not include 7 individuals with no sugars and 20 *S. venustum* used in preliminary trials.

Figure 11 Sugar combinations of (a) female and (b) male black flies from early and late sweep samples of a tamarack stand. Sugar combinations are as follows: A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations), D (Sta combinations) and E (Suc / Tur, and / or Raf combinations). Numbers in brackets above bars indicate the number of flies analysed by T.L.C.



(11a) Sugar profiles of female black flies from early and late sweep samples.



(11b) Sugar profiles of male black flies from early and late sweep samples.

detected from early samples and 28.1% from late samples. Chi square analysis was performed for male and female flies in profiles C and D (i.e., presence of stachyose) versus flies in profiles A, B and E (i.e., absence of stachyose) for early and late samples (Table 4). The frequency of stachyose occurrence differed significantly between females and males from late samples (X^2 = 18.73, df = 1, P< .001), with male flies being more likely than female flies to contain stachyose. The frequency of stachyose occurrence differed significantly between flies from early and late samples (X^2 = 7.38, df = 1, .005 < P < .01), with flies from early samples being more likely than flies from late samples to contain stachyose.

(2) Car Trap Study

The male and female black flies from morning and evening collections corresponding to the five sugar profiles are presented in Table 5. The proportion of flies corresponding to profiles A, B, C, D and E are: 33.8%, 28.1%, 6.3%, 5.6% and 26.3%, respectively for females and 55.6%, 21.9%, 4.4%, 1.3% and 16.9% for males. It was found that 27.5% (44 / 160) of male and 40.0% (64 / 160) of female black flies contained melezitose and / or stachyose (i.e., profiles B, C and / or D) sugars (Table 5; Figure 12 a and b).

Chi-square analysis was performed for male and female flies in categories B, C and D (i.e., presence of melezitose and / or stachyose) versus flies in categories A and E (i.e., absence of melezitose and stachyose) for morning and evening samples. The number of male black flies containing sugars in categories B, C and D versus A and E differs significantly from that of female flies (Table 6; X^2 = 5.05, 0.01 < P < 0.025). The frequency of melezitose and / or stachyose occurrence differed significantly between females and males from morning samples (Table 6; X^2 = 3.87, 0.025 < P < 0.05) but not evening samples. There were no significant differences, with respect to sugar composition, between morning and evening samples, for males or females.

Table 4 Chi - square analysis of female and male black flies containing stachyose sugar combinations (i.e., profiles C and D) versus flies not containing stachyose (i.e., profiles A, B and E), from early and late sweep collections of tamarack.

Comparison			X^2	Probability
Early Female	vs.	Late Female	3.15	$0.05 < P < 0.10$
Early Male	vs.	Late Male	1.21	$0.25 < P < 0.50$
Early (F & M)	vs.	Late (F & M)	7.38	$0.005 < P < 0.01$*
Early Female	vs.	Early Male	1.93	$0.10 < P < 0.25$
Late Female	vs.	Late Male	18.73	$P < 0.001$*
Female (E & L)	vs.	Male (E & L)	24.13	$P < 0.001$*

* Significant ($X^2_{0.05,\ df=1} = 3.841$) Cochran corrected Chi-square

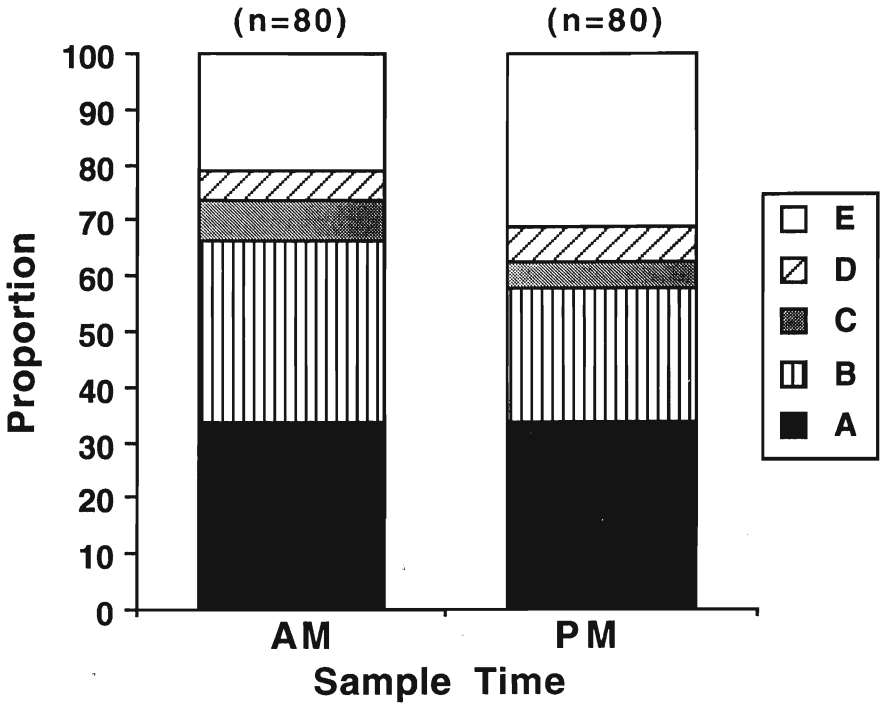
Table 5 Numbers of male and female *S. venustum*[1] containing sugar combinations conforming to each of 5 profiles from AM and PM car trap samples.

	Sugar Profiles*					
Time / Sex	A	B	C	D	E	Total
AM Female	27	26	6	4	17	80
AM Male	45	19	4	0	12	80
AM Total	72	45	10	4	29	160
PM Female	27	19	4	5	25	80
PM Male	44	16	3	2	15	80
PM Total	71	35	7	7	40	160
Overall Total	143	80	17	11	69	320

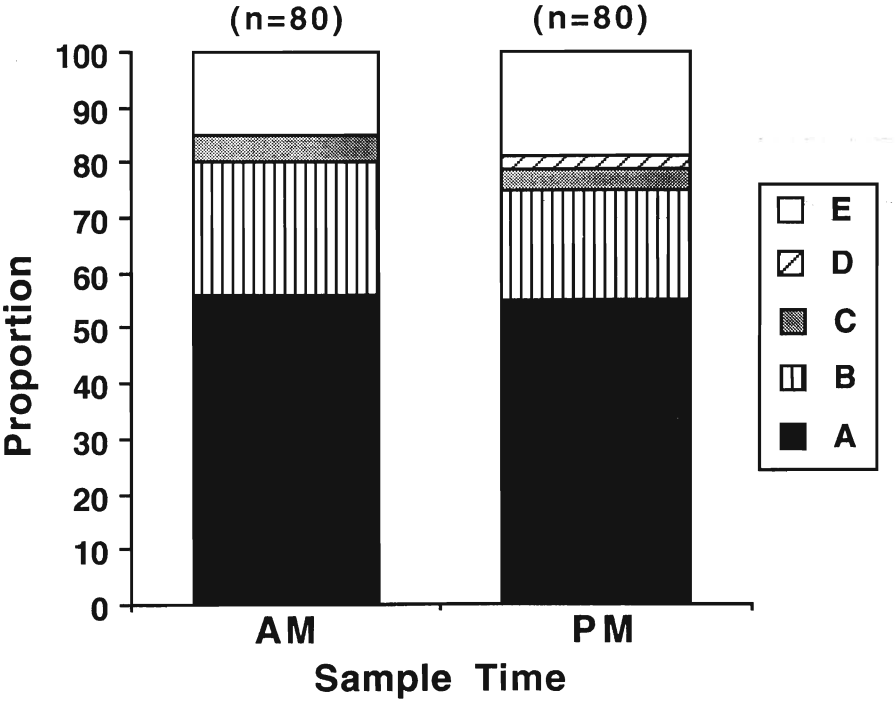
* A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations) D (Sta combinations) and E (Suc / Tur, and / or Raf combinations).

[1] It is possible that a few *S. rostratum* were included in this analysis.

Figure12 Sugar combinations of (a) female and (b) male *S. venustum* from AM and PM collections of Davies' Bog, air field, deciduous and coniferous habitats. Sugar profiles are as follows: A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations), D (Sta combinations) and E (Suc / Tur, and / or Raf combinations). Numbers in brackets above bars indicate the number of flies analysed by T.L.C.



(12a) Sugar profiles of female black flies from AM and PM sample times.



(12b) Sugar profiles of male black flies from AM and PM sample times.

Table 6 Chi - square analysis of female and male *S. venustum* containing stachyose and / or melezitose sugar combinations versus flies not containing stachyose and / or melezitose, from AM and PM car trap samples.

Comparison			X^2	Probability
AM Female	vs.	PM Female	1.28	$0.25 < P < 0.50$
AM Male	vs.	PM Male	0.03	$0.75 < P < 0.90$
AM (F & M)	vs.	PM (F & M)	1.13	$0.25 < P < 0.50$
AM Female	vs.	AM Male	3.87	$0.025 < P < 0.05$*
PM Female	vs.	PM Male	1.06	$0.25 < P < 0.50$
Female (AM & PM)	vs.	Male (AM & PM)	5.05	$0.01 < P < 0.025$*

* Significant ($X^2_{0.05,\ df=1} = 3.841$) Cochran corrected Chi-square

(3) Four Habitat Study

Comparisons of AM versus PM sugar profiles for *S. venustum* can be seen in Figures 13a and 13b, and for species other than *S. venustum*, in Figures 14a and 14b. In the air field, deciduous and coniferous habitats, in both the AM and PM samples, the majority of *S. venustum* flies contained sugar combinations belonging to profiles A and B, whereas at Davies Bog, this is not the case (Figures 13a and 13b). There were no significant differences among sites or between AM and PM collections for species other than *S. venustum* (Figures 14a and 14b).

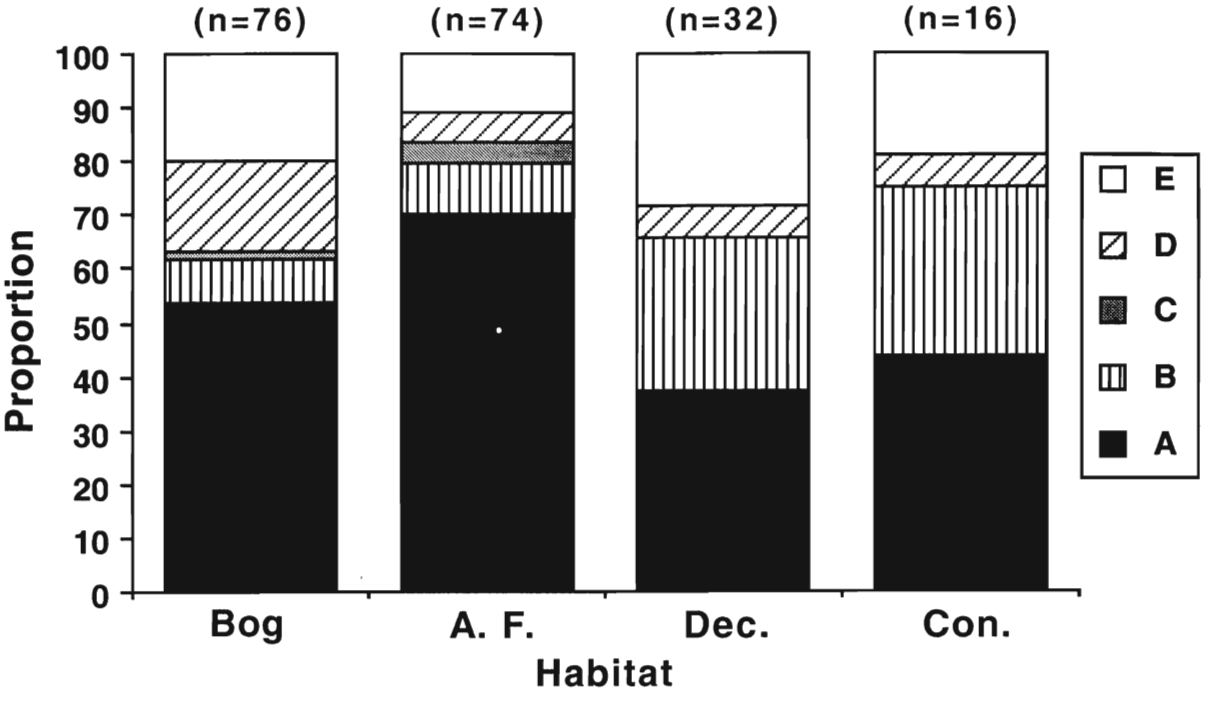
Davies Bog

The number of *S. venustum* and of species other than *S. venustum* corresponding to these five profiles are shown (Table 7). The proportion of *S. venustum* from Davies Bog corresponding to categories A, B, C, D and E are: 42.7%, 14.7%, 1.9%, 17.8% and 22.9% and for species other than *S. venustum* 27.7%, 21.2%, 3.7%, 8.8% and 38.7%. It was found that 34.4% (54 / 157) of *S. venustum* and 33.6% (46 / 137) of species other than *S. venustum* contained melezitose and / or stachyose sugar combinations.

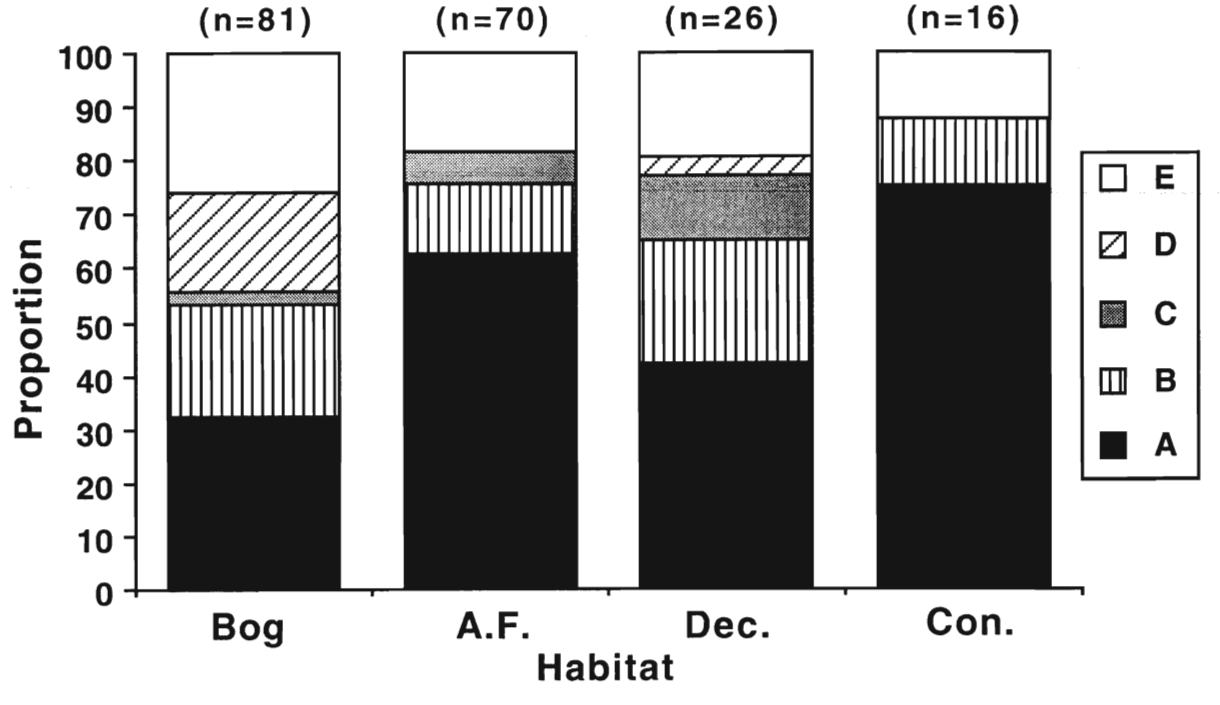
Air Field

The number of *S. venustum* and of species other than *S. venustum* corresponding to these five profiles are shown (Table 7). The proportion of flies corresponding to categories A, B, C, D and E are: 66.7%, 11.1%, 4.9%, 2.8% and 14.6% for *S. venustum* and 45.2%, 8.3%, 9.5%, 7.1% and 29.8% for species other than *S. venustum*. It was found that 18.8% (27 / 144) of *S. venustum* and 25.6% (21 / 84) of species other than *S. venustum* contained melezitose and / or stachyose sugar combinations.

Figure 13 Sugar combinations of *S. venustum* from (a) AM and (b) PM collections of Davies Bog, air field, deciduous and coniferous habitats. Sugar combinations are as follows: A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations), D (Sta combinations) and E (Suc / Tur, and / or Raf combinations). Numbers in brackets above bars indicates the number of flies analysed by T.L.C.

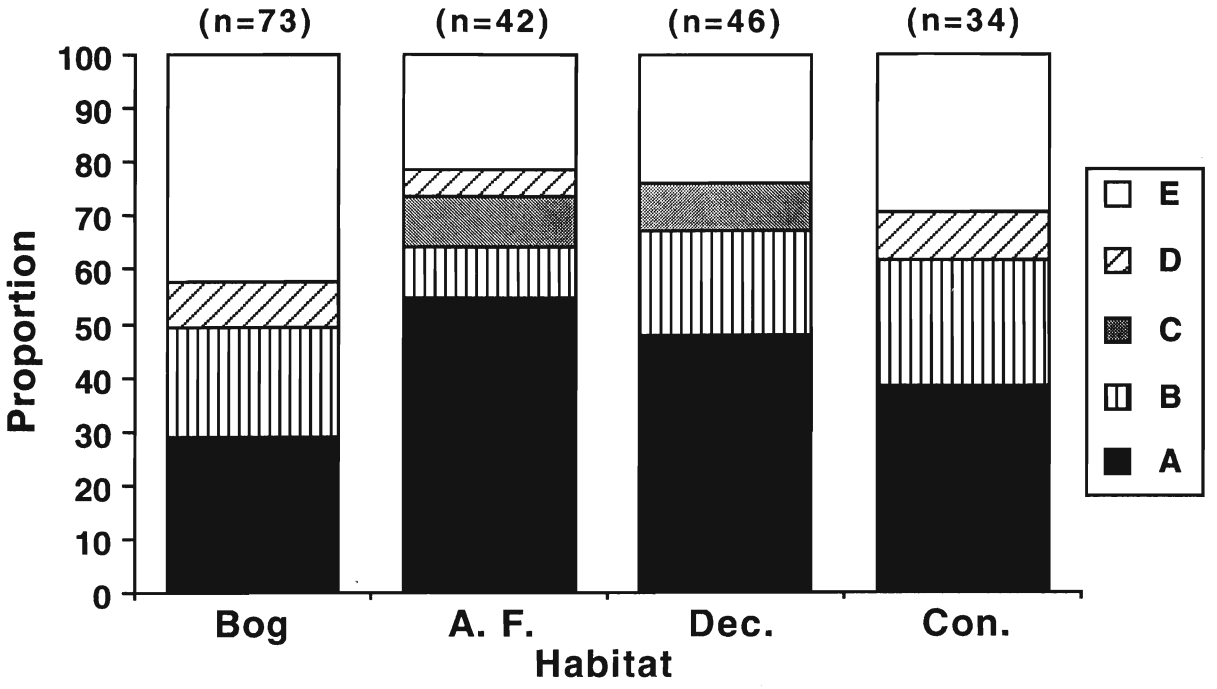


(13a) Sugar profiles of S. venustum from AM collections.

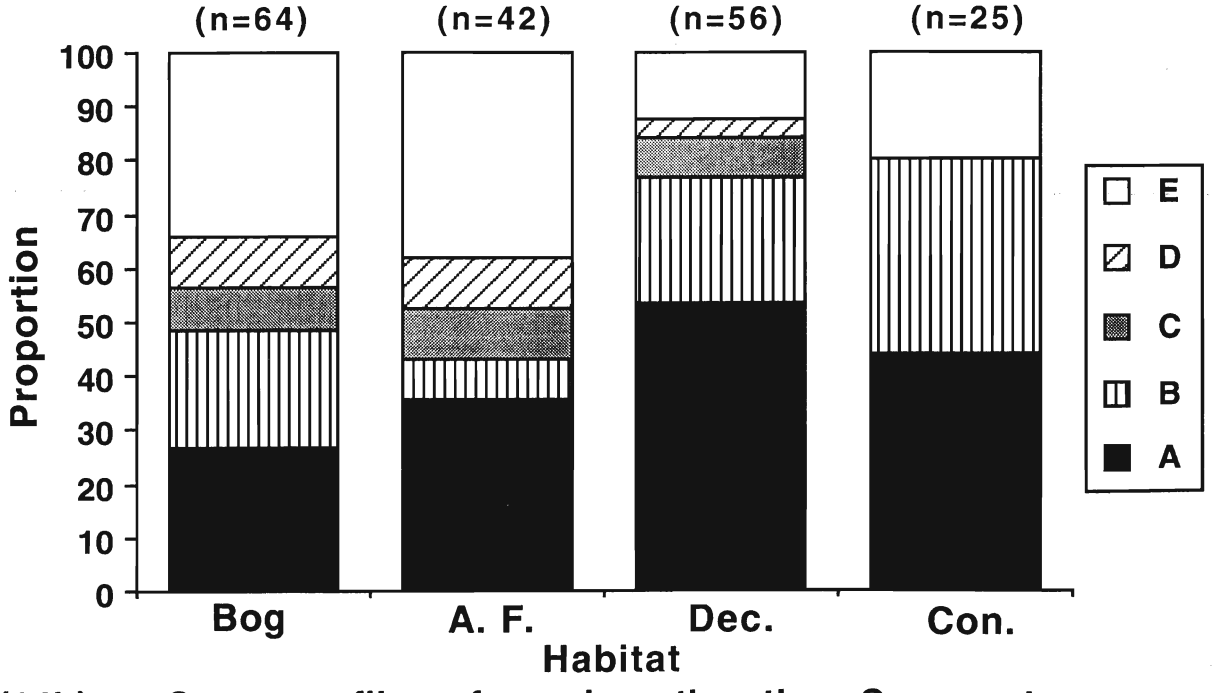


(13b) Sugar profiles of S. venustum from PM collections.

Figure 14 Sugar combinations of species other than *S. venustum* from (a) AM and (b) PM collections of Davies Bog, air field, deciduous and coniferous habitats. Sugar combinations are as follows: A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations), D (Sta combinations) and E (Suc / Tur, and / or Raf combinations). Numbers in brackets above bars indicates the number of flies analysed by T.L.C.



(14a) Sugar profiles of species other than S. venustum from AM collections.



(14b) Sugar profiles of species other than S. venustum from PM collections.

Table 7 Numbers of female *S. venustum* and other species containing sugar combinations conforming to each of 5 profiles, from Davies Bog, air field, deciduous and coniferous habitat samples (AM and PM samples are combined).

	Sugar Profiles*					
	A	B	C	D	E	Total
S. venustum						
Davies Bog	67	23	3	28	36	157
Air Field	96	16	7	4	21	144
Deciduous	23	15	3	3	14	58
Coniferous	19	7	0	1	5	32
Total	205	61	13	36	76	391
Other species						
Davies Bog	38	29	5	12	53	137
Air Field	38	7	8	6	25	84
Deciduous	52	22	8	2	18	102
Coniferous	24	17	0	3	15	59
Total	152	75	21	23	111	382
Overall Total	357	136	34	59	187	773

* A (Fru, Glc only), B (Mez combinations), C (Sta and Mez combinations) D (Sta combinations) and E (Suc / Tur, and / or Raf combinations).

Deciduous Habitat

The number of *S. venustum* and of species other than *S. venustum* corresponding to these five profiles are shown (Table 7). The proportion of flies corresponding to categories A, B, C, D and E are: 39.7%, 25.9%, 5.2%, 5.2% and 24.1% for *S. venustum* and 51.0%, 21.6%, 7.8%, 2.0% and 17.7% for species other than *S. venustum*. It was found that 36.2% (21 / 58) of *S. venustum* and 31.4% (32 / 102) of species other than *S. venustum* contained melezitose and / or stachyose sugar combinations.

Coniferous Habitat

The number of *S. venustum* and of species other than *S. venustum* corresponding to these five profiles are shown (Table 7). The proportion of flies corresponding to categories A, B, C, D and E are: 59.4%, 21.9%, 0%, 3.1% and 15.6% respectively for *S. venustum* and 40.7%, 28.8%, 0%, 5.1% and 25.4% for species other than *S. venustum*. It was found that 25.0% of *S. venustum* and 33.9% of species other than *S. venustum* contained melezitose and / or stachyose sugar combinations.

Habitat Chi-Square analyses

Chi-square analysis was performed in order to determine whether the frequency of sugars differed between *S. venustum* individuals collected from each of the 4 habitats (Table 8). A test was similarly conducted to determine whether the frequency of sugars differed between species other than *S. venustum* individuals collected from each of the 4 habitats (Table 8). A third set of tests was performed by combining all species from each of the 4 habitats (Table 8). For these tests sugar profiles B, C and D were grouped (i.e., to indicate the presence of melezitose

Table 8 Chi - square analysis of black flies containing stachyose / melezitose sugar combinations (profiles B, C and D) versus flies not containing stachyose / melezitose (profiles A and E), from habitat samples.

Comparison	X^2	Probability
S. venustum		
(1) Bog vs. Dec. vs. Con. vs. A.F.	11.34	$0.01 < P < 0.025$*
(2) Bog vs. Dec. vs. Con.	1.28	$0.50 < P < 0.75$
(3) Bog, Dec., Con. vs. A.F.	9.19	$0.001 < P < 0.005$*
Other species		
(4) Bog vs. Dec. vs. Con. vs. A.F.	2.06	$0.25 < P < 0.50$
All species		
(5) Bog vs. Dec. vs. Con. vs. A.F.	11.75	$0.005 < P < 0.01$*
(6) Bog vs. Dec. vs. Con.	0.33	$0.75 < P < 0.90$
(7) Bog, Dec., Con. vs. A.F.	11.34	$P < 0.001$*

* Significant

(1), (4) and (5) d.f. = 3 ($X^2 = 7.815$)

(2) and (6) d.f. = 2 ($X^2 = 5.991$)

(3) and (7) d.f. = 1 ($X^2 = 3.841$) Cochran Corrected Chi - Square

and / or stachyose), as were A and E (i.e., to indicate the absence of melezitose and stachyose). It was found that the frequency of melezitose / stachyose occurrence in *S. venustum*, from the air field, differed significantly from the other three habitats ($X^2_{0.05, 1} = 9.19$, $0.001 < P < 0.005$).

(C) Behavioural Observations

(1) Tamarack Study

A sequence of photographs from videotaped footage is presented, demonstrating that male and female black flies (*S. venustum*) will feed on homopteran honeydew. After being introduced into the petri dish, male and female black flies quickly began to aggregate on the tamarack branches. While moving over the surface of the branches the flies were constantly palpitating the substrate with the tarsi of their fore legs. At this point the video camera was trained upon a droplet of honeydew protruding through a mass of eggs surrounding a single adult homopteran (Figure 15a). A male black fly then approached and brushed the droplet with its foretarsus (Figure 15b), then quickly turned in the direction of the honeydew droplet and began to feed (Figure 15c). Soon after, a female black fly also began to feed from the same droplet (Figure 15d). Three more female black flies began feeding, by which point the honeydew droplet was completely covered by flies (Figure 15e). After approximately two minutes the aggregation of flies began to move away leaving no trace of the honeydew droplet (Figure 15f).

During additional trials, it was observed that the black flies were able to ingest freshly excreted honeydew (i.e., extending as a fluid droplet from the anus of a homopteran) or older honeydew (i.e., appearing as glossy or shiny areas on the branches and needles of the tamarack). When honeydew was encountered, a black fly would stop and apply its mouthparts to the surface of the droplet. The abdomen of the black fly could be observed becoming distended when larger droplets were consumed.

Figure 15 a, b, c Sequence of photographs depicting the ingestion of homopteran honeydew, by black flies, from tamarack branches. See text for additional comments.


MAY 21 '93
AM 12:41

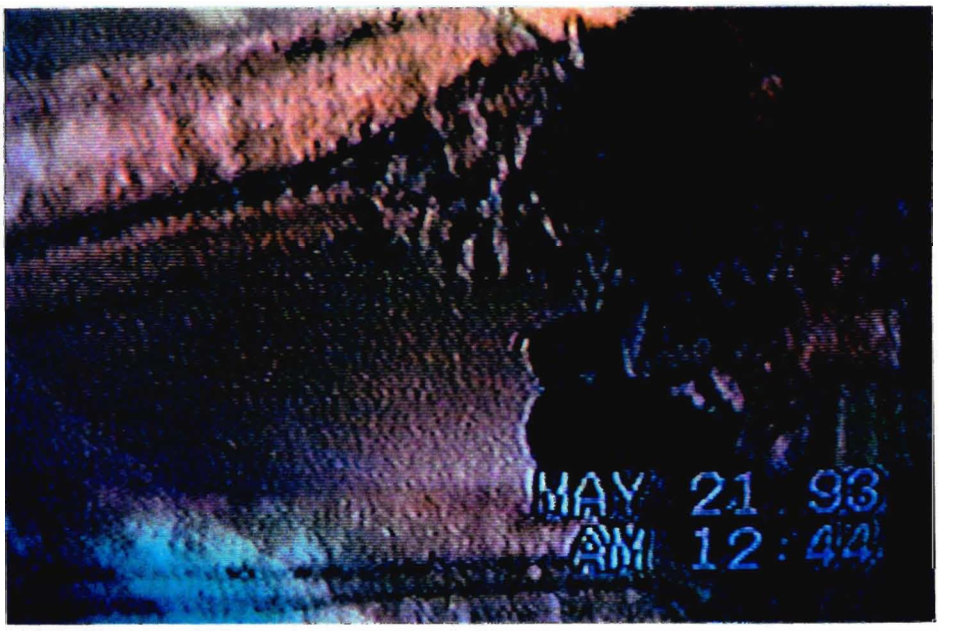

MAY 21 '93
AM 12:41


MAY 21 '93
AM 12:41

Figure 15 d, e, f Sequence of photographs depicting the ingestion of homopteran honeydew, by black flies, from tamarack branches. See text for additional comments.


MAY 21 93
AM 12:41


MAY 21 93
AM 12:42


MAY 21 93
AM 12:44

Seasonal Trends

This is based on the number of sugar combinations, present on each of 8 calendar week dates from car trap samples and the four habitat study. The correlation investigated is between the time of season and the number of sugar combinations present in flies tested by T. L. C. from the four habitats and the car trap samples. These data are presented graphically in Figures 16 (car trap), 17 (Davies Bog), 18 (air field), 19 (deciduous habitat) and 20 (coniferous habitat).

The r^2 values are: car trap (0.498), Davies Bog (0.239), air field (0.323), deciduous habitat (0.145) and coniferous habitat (0.630). The critical value for rejecting the null of no linear correlation is 0.707 (n=8, P=0.05). The car trap correlation coefficient value is r=0.706. The coniferous correlation coefficient value (r=0.794) does reject the null hypothesis (i.e., positive correlation is present) indicating an increase in the number of sugar combinations present in the midguts of flies as the season progresses from mid-late spring to early - mid summer. The deciduous site indicates almost a straight horizontal line across the graph (i.e., no correlation).

Figure 16 Graph of the number of sugar combinations found in black flies collected from car trap samples on 8 sample days.

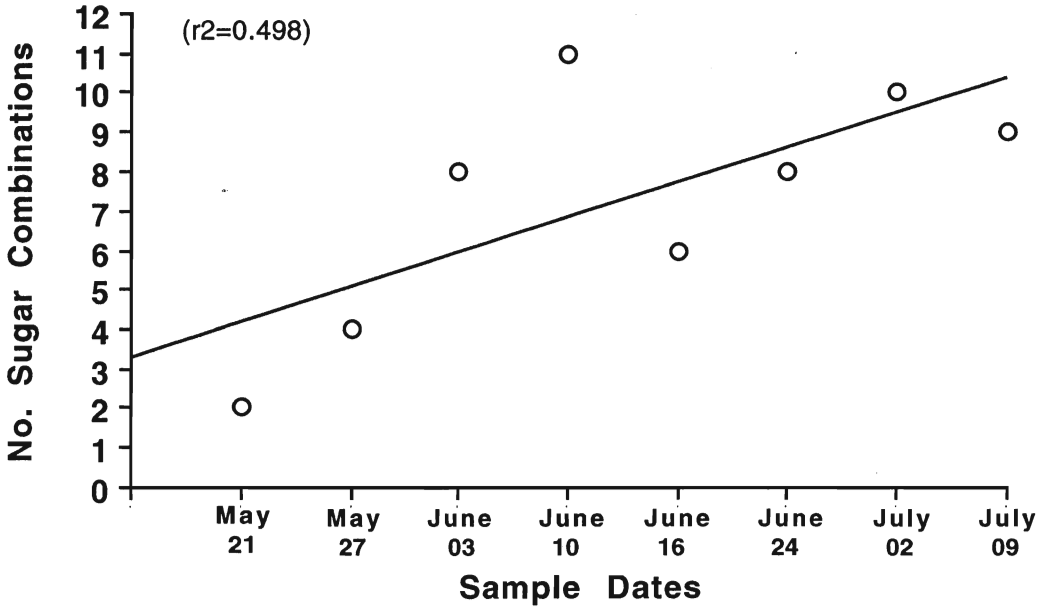

(r2=0.498)
No. Sugar Combinations
0
1
2
3
4
5
6
7
8
9
10
11
12
May 21
May 27
June 03
June 10
June 16
June 24
July 02
July 09
Sample Dates

Figure 17 Graph of the number of sugar combinations found in black flies collected from Davies Bog samples on 8 sample days.

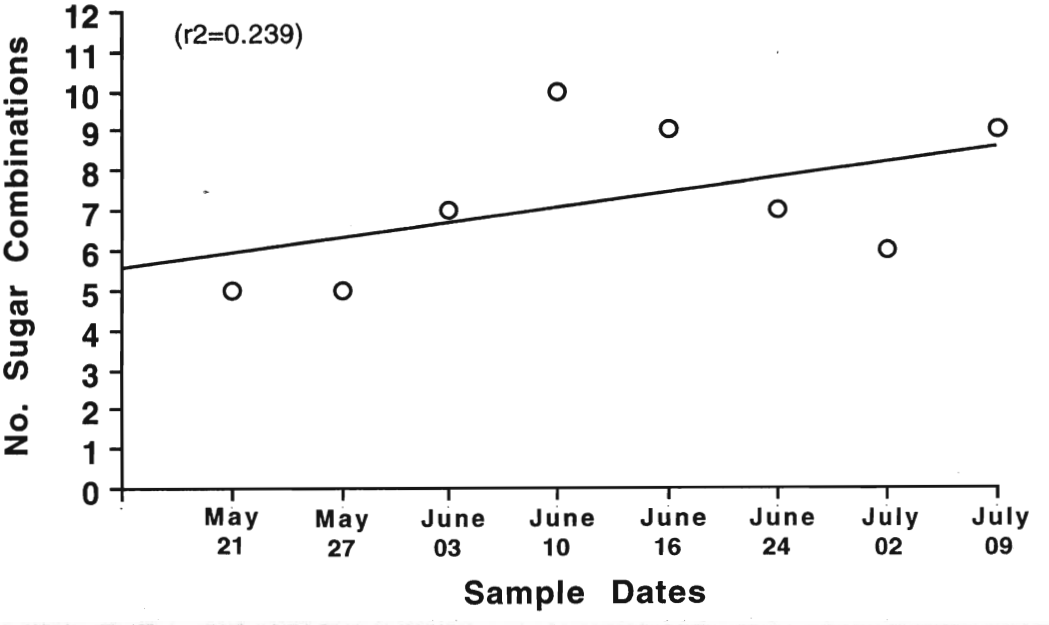

(r2=0.239)
No. Sugar Combinations
0
1
2
3
4
5
6
7
8
9
10
11
12
May 21
May 27
June 03
June 10
June 16
June 24
July 02
July 09
Sample Dates

Figure 18 Graph of the number of sugar combinations found in black flies collected from air field samples on 8 sample days.

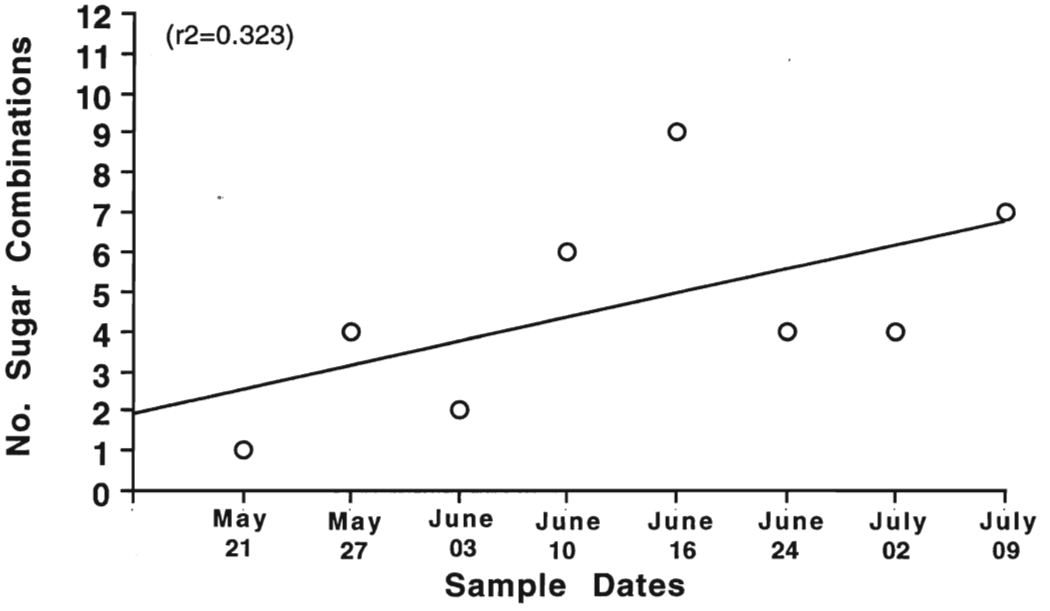
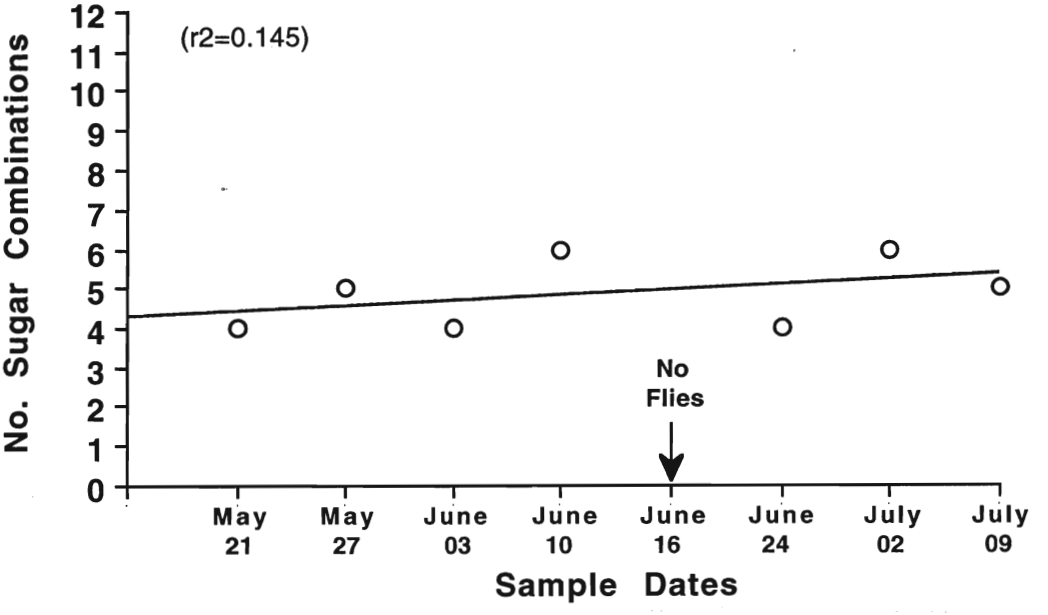
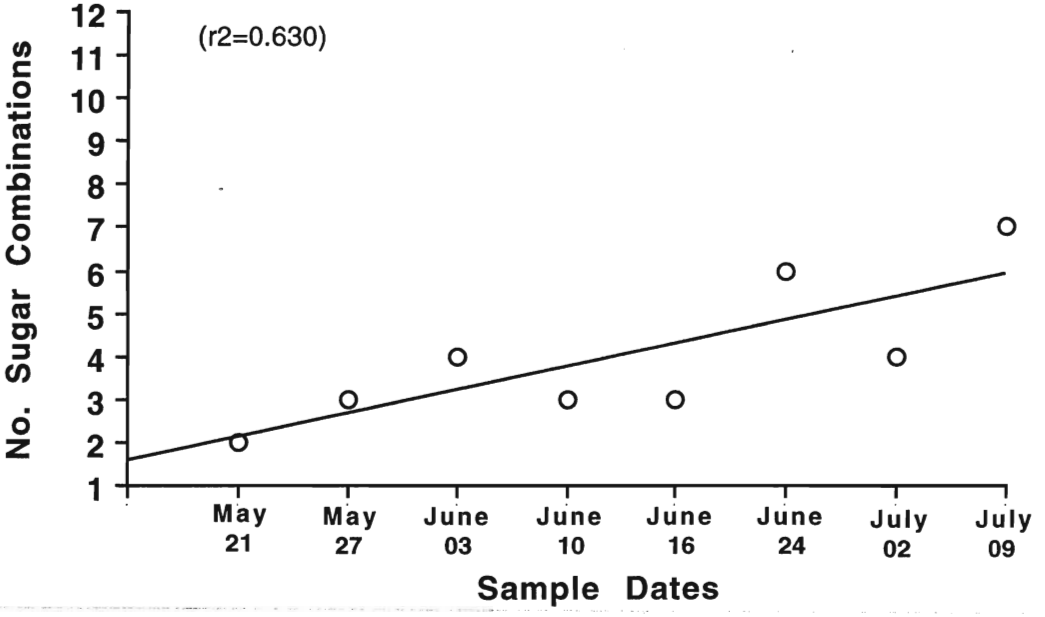

(r2=0.323)
No. Sugar Combinations
0
1
2
3
4
5
6
7
8
9
10
11
12
May 21
May 27
June 03
June 10
June 16
June 24
July 02
July 09
Sample Dates

Figure 19 Graph of the number of sugar combinations found in black flies collected from deciduous habitat samples on 8 sample days.



Note: analysis does not include date on which no flies were collected.

Figure 20 Graph of the number of sugar combinations found in black flies collected from coniferous habitat samples on 8 sample days.


(r2=0.630)
No. Sugar Combinations
12
11
10
9
8
7
6
5
4
3
2
1
May 21
May 27
June 03
June 10
June 16
June 24
July 02
July 09
Sample Dates

Discussion

Stachyose and Melezitose as Indicators of Honeydew Feeding

The use of the sugar melezitose as an indicator of honeydew feeding seems to be generally accepted. Within the literature, there seems to be strong support for the hypothesis that melezitose is synthesized in the guts of aphids (Hudson, 1946; Bacon and Dickinson, 1955; Bacon and Dickinson, 1957). There are several studies which demonstrate the presence of melezitose in honeydew, but not in the phloem of the plants upon which the homopterans were feeding (Mittler, 1958; Auclair, 1963; Hussain *et al.*, 1974; Byrne and Miller, 1990). For this reason, the presence of melezitose in the guts of black flies may be a very strong indicator that these flies were feeding from a honeydew source. Of the 1287 black flies tested in this study it was found that 289 (22.5%) contained melezitose.

The sugar stachyose, which was found in the honeydew of adelgid homopterans infesting the tamarack, may have been present in the phloem of the tamarack (although phloem fluid was never analysed). The homopterans may simply have excreted the surplus carbohydrates (including stachyose) so that they remain little changed after passing through the gut. This should not detract from the assertion that black flies, especially those collected from the tamarack, were feeding from the honeydew. Of the 1287 black flies tested in this study, 209 (16.6%) contained stachyose.

There may be several possible methods by which plant phloem material may reach the exterior of plants, such as from wounds caused by other organisms feeding upon the plant material, or simply from branches being broken off in high winds or a number of other such means. However, the large quantities of sugars that reach the outside of plants due to the feeding of homopterans should not be overlooked, especially in light of the findings regarding the tamarack. The suggestion that black flies are able to bite into plant tissues to feed on the phloem

directly (Hunter, 1977) may be exceptional, as the mouthparts of all males and of females of some black fly species (e.g., *Cnephia dacotensis*) are atrophied and would be of little use in biting through plant tissues.

In the present study, the sugar turanose could not be used to indicate the presence of honeydew in the absence of melezitose, as turanose and sucrose migrated to a similar level and could not easily be distinguished using the urea reagent. Turanose has been used to indicate the presence of melezitose and therefore honeydew in sand fly studies (Moore *et al.*, 1987; Wallbanks *et al.*, 1990). Based on some preliminary sugar feeding studies, some questions arise about the use of this sugar, in the absence of melezitose, to indicate honeydew feeding. In preliminary tests, black flies were reared from pupae, in the laboratory. When tested by T. L. C., after being kept in an enclosure without sugars, no sugars could be detected in the flies. Several batches of black flies were fed sucrose, only, for 1-3 hours. When these flies were tested by T. L. C. (using the D. A. P. A. reagent) four sugars were visible: fructose, glucose, sucrose, and an additional sugar (presumably a disaccharide). This additional sugar had an hRf value similar to that of sucrose / turanose, but the colour of the spot on the T. L. C. plate was pink. When sucrose and turanose standards were developed using the D. A. P. A. reagent, the colours were yellow / green for sucrose and pink for turanose. None of the other standards used were similar in colour to turanose and only sucrose migrates to a similar level. Yang and Davies (1968) found that the sugars fructose, glucose and sucrose and an unknown sugar (an "oligosaccharide") which migrated to a level just below sucrose were detected in the T. L. C. analysis of a solution containing sucrose and black fly midgut tissue. They concluded that the gut enzyme invertase may act on sucrose to synthesize another oligosaccharide.

It is suggested, therefore, that future studies are needed to establish whether or not a sugar such as turanose or some other oligosaccharide can be synthesized in the guts of black flies. Because of the importance placed on the sugar turanose

as an indicator of honeydew feeding, it is important to more clearly determine the action of the gut enzymes on sugars such as sucrose. It would be possible to determine whether or not turanose was present in flies after they had been fed sucrose, by analysing the gut contents using T. L. C. and a reagent called T. Z. B. (tetrazole blue - sodium hydroxide) (Damonte *et al.,* 1971). This reagent does not react with sucrose, glucose or melezitose, but will react with turanose to produce an intense "lilac" colour (Damonte *et al.,* 1971). The sugar fructose is also detected, but this sugar migrates at a different rate than does turanose.

Using the urea reagent to develop sugars from blueberry nectar, it was found that 2 combinations were present: combination 1 (fructose, glucose only) and combination 2 (frucose, glucose and sucrose / turanose). The proportions of the black flies containing these combinations from the tamarack site, car trap samples, Davies Bog, air field, deciduous and coniferous habitats are: 43.3%, 59.1%, 58.5%, 77.2%, 63.8% and 67.0%, respectively. These numbers may indicate the maximum number of black flies, tested by T. L. C., that may have fed on blueberry nectar. There are two main reasons to regard these proportions as overestimates of blueberry nectar feeding. First, the three sugars fructose, glucose and sucrose are considered to be the dominant and often the only sugars found in a variety of plant nectars (Baker and Baker, 1983; Freeman and Wilken, 1987; Freeman *et al.*, 1991). Second, sugars such as raffinose, melezitose and stachyose, which were not found in samples of blueberry nectar, may be broken down, by digestion, into these three sugars. The sensitivity of the T. L. C. methods used in this study may not have been sufficient to detect trace amounts of sugars remaining after digestion. In addition, small amounts of sugars may have been present due to small sugar meals having been ingested, or perhaps due to low concentrations of these sugars within the sugar source.

Importance of Analysing Sugar Meals in Male Black Flies

The ratio of male to female black flies increases, relative to vegetation sweep samples of the other four habitats, in the presence of a sugar source (in this case *Larix laricina* infested with honeydew - producing homopterans of the family Adelgidae). It may be more convincing to have males (rather than females) in a sweep sample of vegetation, to indicate that a potential sugar meal source is nearby. In the study by Davies and Peterson (1956) it is suggested that black flies may obtain sugars form the nectar of blueberries. However in that study a single male and 227 female black flies were captured. It is likely that the female black flies were attracted more to the researchers, as potential blood meals, than to the blueberry flowers. Male black flies do not require blood meals and would therefore provide greater evidence that they were present within the vegetation in order to obtain a nectar meal. Since so few males were found in the four habitat study associated with the Ericaceous shrubs, it is probable that they are gleaning their sugar meals elsewhere.

Males Versus Females (Tamarack Study)

In the laboratory, both female and male black flies readily ingest honeydew from homopterans infesting tamarack. Based on laboratory observations, the honeydew can be ingested when freshly excreted (i.e., in a liquid state) or when 'older' (i.e., relatively dry). As evidenced by chromatographic analysis of sugars, male and female black flies will also consume honeydew, from homopterans infesting tamarack, in the field.

The capture of male black flies not involved in mating swarms and located among the tamarack branches during early and late sweeps suggests that these individuals were in fact associated with the trees. In late sweeps the total number

of male black flies (n = 22) is lower than in early sweeps (n = 34) suggesting that the human collectors do not attract male flies. The number of female flies (n = 32) in early sweeps approximately equals that of the males (n = 34), and therefore indicates that these females were within the vicinity of the tamarack before the collectors appeared. In the late sweeps, the number of female flies is approximately 7 times greater than the number of males, most likely as the collectors represent potential blood meals.

With respect to the number of flies containing stachyose, fewer flies contained this sugar in early samples (n = 32) than in late samples (n=50), suggesting that the latter flies were missed during the early sample. This is understandable as only the bottom 3.0 - 3.5 m of the 10 m tall trees were sampled. A greater number of female flies contained stachyose in the later samples (n = 34) than early samples (n = 12) perhaps indicating that the females were resting or feeding higher within the canopy or were in the vicinity of the trees during the first sample and were attracted to the collectors by the time the second sample was taken. However, the proportion of females containing stachyose from late samples is reduced compared to early samples indicating that many more female flies in the late samples were not associated with the tamarack, but arrived from other locations.

It is unlikely that the 16 sugar profiles in Appendix 26 each represents a separate food source for the 194 individuals tested. It is possible that in some of the individuals, sugars that do not appear may simply be in lower concentrations than the T. L. C. methods employed could detect, resulting in different sugar combinations. These low concentrations may occur if a black fly takes only a small sugar meal, or if some sugars were broken down by digestion in the flies before they were frozen. It has been found that sugars in honeydew such as raffinose and stachyose may be metabolized by the enzyme invertase present in fungi and bacteria growing on the honeydew (Davis *et al.*, 1993). For this reason, a single

source of honeydew such as the adelgid honeydew found at the tamarack site may provide a variety of sugar combinations for flies feeding from this source at different times.

In addition, honeydew sugars can also vary depending on the species of homopteran present and on the type of plant being infested (Hendrix *et al.*, 1992). The trisaccharide melezitose is a characteristic sugar in a number of honeydews (Hudson and Sherwood, 1920; Hudson, 1946; Byrne and Miller, 1990) and has been used to indicate when sand flies (Diptera: Psychodidae) have fed from a honeydew source (MacVicker *et al.*, 1990). In the present study, however, melezitose was not detected in the honeydew of homopterans infesting tamarack. It is interesting, therefore, that several female flies (n = 18) in the late samples and a few from early tamarack samples (n = 3) contained this sugar, perhaps indicating that these flies previously fed from a different honeydew source.

By considering the flies that contained melezitose and / or stachyose sugar combinations (profiles B, C and D) it is revealed that 53.6% of the black flies that were caught at the homopteran-infested tamarack site had fed from a honeydew source.

Males Versus Females (Car Trap Study)

It was found that both male and female flies (*S. venustum*) contain the sugars melezitose and stachyose, indicating that both sexes feed on homopteran honeydew. It was found that the frequency in which these sugars occurred differed significantly between male and female *S. venustum* from AM samples, but not PM samples. During the dissections of the male and female flies, it was noted that the crop size of the female black flies was often larger than that of the males. It would be reasonable to assume that a larger crop would be more likely to provide sufficient quantities of various sugars to be detected using the T. L. C. methods

employed. However, this does not explain why such a difference would only be present from AM samples. It is possible that some behavioural differences which occur between male and female black flies may result in differences in the types of sugars contained within them. It is known that females of most black fly species must expend energy in order to search for a suitable blood host. They also must search for suitable oviposition sites. The male fly does not need to engage in either of these activities. Therefore, it is possible that the movements of female black flies may take them into habitats, in which the variety and abundance of sugar sources differs from those to which the male fly is exposed.

Species Composition (Four Habitat Study)

In the four habitat study, as in the tamarack study, an attempt was made to sweep the vegetation (as opposed to sweeping the air around the collector). If males gain a lot of their sugars from the nectaries of flowering plants, one would assume that by sweeping the vegetation reasonably high numbers of males would be collected. In the air field and in Davies Bog where flowering Ericaceous shrubs were abundant, males simply were not collected in significant numbers.

Males versus Females (Four Habitat Study)

Among the four habitats sampled, the greatest number of flies occurred in the Davies Bog habitat. The second highest abundance of flies occurred in the air strip. The Davies Bog and air field habitats are more open than the coniferous and deciduous habitats. As indicated by Fallis (1964), vegetation may restrict the flight range or movement of black flies.

The number of black fly species identified, from the flies used in T. L. C. analysis, varied between Davies Bog (9 species), the air field (10 species), the

deciduous habitat (11 species) and the coniferous habitat (12 species), as did the number of individuals of each species. The species *St. mutata* and *P. fontanum* were found from collections in the deciduous site but were absent in Davies Bog and the air field, and were only present in low numbers in the coniferous site. This may be explained by the presence of a stream near the deciduous habitat collection site, in which *Prosimulium spp.* and *St. mutata* larvae were found. The high number of *S. quebecense* in the coniferous habitat may support the hypothesis that this species tends to feed on birds within the canopy of trees (Bennett, 1960).

Comparisons of the total number of male flies present in samples from the four habitats (n = 7) as opposed to the tamarack stand (n = 56) is interesting for two reasons. First, although the sampling effort was higher in the four habitat study (64, fifteen minute samples) as compared to the tamarack study (12, six minute samples), more males were collected from the tamarack site. Second, as male flies are not likely to be influenced by the presence of a possible blood - host (i.e., the collector) they may be associated with vegetation in order to obtain sugar - meals. As the tamarack were known to be infested with honeydew producing homopterans, it is probable that male flies were present as a result of this sugar source.

Seasonal Differences

The variety of sugar sources available to black flies appears to increase between May 21 and July 09 in Algonquin Provincial Park. This is based on the number of sugar combinations present on each of 8 calendar week dates from car trap samples and the four habitat study.

The increase in the number of sugar combinations, as the season progresses, within black flies in the coniferous habitat is interesting. It is possible

that as the number of flies tested by T. L. C. increases, the number of sugar combinations found with flies also increases. It is interesting, therefore, that the correlation of the number of sugar combinations over time deviated significantly from a zero slope only in the coniferous site, as this site had the fewest flies tested of all sites. In this study the gut contents of 1287 individual black flies were tested by T. L. C. The sugars present were found to occur in 16 different combinations only for the flies from the tamarack site, whereas flies from the car trap and Davies Bog samples contained a total of 14 sugar combinations and flies from the air field and deciduous site contained 12. The fewest number of sugar combinations occurred in flies sampled from the coniferous site in which 9 combinations were identified.

The number of sugar combinations present by calendar week 20 is greater in black flies from Davies Bog (5 combinations) and the deciduous habitat (4 combinations) than in the car trap (2 combinations), air field (1 combination) and the coniferous habitat (2 combinations). Perhaps it is in this respect that the sugar combinations seem less changed (i.e., do not increase as much relatively) throughout the season for Davies Bog and the deciduous habitat. In other words these sites begin the season with a greater variety of sugar combinations. We know that some bog plants (leather leaf) flower very early, and that the deciduous forest flowering plants must start early enough that they are not shaded out by the deciduous trees, once they start leafing out. Perhaps these are some of the factors which are affecting the number of sugar combinations present in these areas.

Conclusions

A variety of organisms are known to feed upon homopteran honeydew. Perhaps the best known example is that of ants that "tend" homopterans in order to obtain honeydew. In return, the homopterans are provided some form of protection by the ants (Kiss, 1981). General surveys have been conducted to determine the range of insects visiting honeydew (Judd, 1978; Schlee, 1977; Gokulpure and Mehra, 1977). A number of studies have focused on more specific groups of insects, such as bumble bees (Batra, 1993), chironomids (Downes, 1974) and horseflies (Schutz and Gaugler, 1989). Until this study, black flies have been absent from the large list of organisms known to ingest honeydew.

Honeydew from homopterans of the family Adelgidae infesting *L. larcina* is readily consumed by male and female black flies under laboratory conditions. In the field, both male and female flies were associated with this source of sugar. The generalization that black flies 'nectar' feed is, therefore, inaccurate as it assumes that only floral sources of sugar are available to simuliids.

Based on the literature and on personal observations, it is apparent that two main sources of carbohydrate energy are readily available to black flies. My research has been directed towards elucidating the extent of non-floral sugar use by black flies. Data collected during the 1993 field season has supported the idea that black flies feed on homopteran honeydew. The question as to whether black flies pollinate blueberries has persisted and is now being regarded as a fact. This research has indirectly addressed this question, by providing evidence that there is at least one vast source of sugar present during early to late spring. It is interesting to note that the peak activity of black flies also corresponds with the period of time in which large quantities of honeydew are being produced by the adelgid homopterans infesting the tamarack. This suggests that blueberry nectar is not the only rich source of carbohydrates available to the black flies in Algonquin Park.

Overall Significance of Findings

If the results from all three studies are considered together, one finds that 34.3% of all flies analysed by T. L. C. tested positive for honeydew (Table 2; sugar profiles B, C and D). Thus, honeydew is an important sugar source that has been largely overlooked by simuliid researchers.

This is the first study in North America to look at sugar sources used by any dipteran in such a rigorous manner, i.e., by using a variety of collection methods, by sampling over the course of the season and by sampling from a variety of habitats.

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Appendix 1 A list of the black fly species occurring in Algonquin Provincial Park, ON. Information from Davies, Peterson and Wood, (1962) unless otherwise indicated (** in Davies and Györkos, 1990).

Prosimulium (Prosimulium) fontanum Syme & Davies, 1958
Univoltine (Mammals), Facultative autogeny for first gonotrophic cycle**
Pupation - End of May (eggs partly developed **)

Prosimulium (Prosimulium) fuscum Syme & Davies, 1958
Multivoltine (2) (Mammals), Facultative autogeny for first gonotrophic cycle**
Pupation - Early May (eggs partly developed**)

Prosimulium (Prosimulium) mixtum Syme & Davies, 1958
Univoltine, (multivol. 2) (Mammals), Anautogenous**
Pupation - Mid April - June

Ectemnia invenusta (Walker, 1848)
Univoltine (Birds), Anautogenous
Pupation - Early - Mid April

Stegopterna mutata (Triploid) all Female
Parthenogenic,
Facultative autogeny for first gonotrophic cycle but dipl. / tripl. not indicated**
Pupation - Late April- Early May-June

Eusimulium (Eusimulium) aureum Fries, 1824
Multivoltine (2) (Birds), Anautogenous
Pupation - Early - Late June

Eusimulium (Nevermannia) vernum Macquart, 1826 *"craigi" "caledonense"*
Univoltine, Multivoltine (Birds), Obligate anautogenous but not specific**
Pupation - June

Eusimulium (Nevermannia) quebecense Twinn, 1936
Univoltine (Birds), Anautogenous
Pupation - May 17 - June 8

Eusimulium (Nevermannia) croxtoni Nicholson & Mickel, 1950
Multivoltine (2) (Birds), Anautogenous
Pupation - Mid - Late May

Eusimulium (Hellichiella) euryadminiculum Davies, 1949
Univoltine? (Loons), Obligate anautogenous **
Pupation - Late April - Early May

Simulium (Psilozia) vittatum Zetterstedt, 1838
Multivoltine (Mammals), Facultative autogeny for first gonotrophic cycle**
Pupation - Early May (eggs partly developed**)

Simulium (Simulium) decorum Walker, 1848
Multivoltine, Facultative autogeny for first gonotrophic cycle **
Pupation - Mid May (eggs partly developed**)

Simulium (Simulium) parnasum Malloch, 1914
Univoltine (Mammals), Anautogenous
Pupation - Mid June

Simulium (Simulium) tuberosum (complex) (Lundstroem, 1911)
Multivoltine (3) (Mammals), Obligate anautogenous**
Pupation - Late May - Early June

Simulium (Simulium) venustum Say, 1823
Multivoltine (Mammals), Obligate anautogenous**
Pupation - Mid - Late May

Simulium (Simulium) truncatum (Lundstroem, 1911)
Univoltine, Anautogenous in USSR (Usova, 1961 in Crosskey, 1990)
Pupation - Mid - Late May

Simulium (Simulium) rostratum (Lundstroem, 1911)
Multivoltine (2-3), Anautogenous in USSR (Usova, 1961 in Crosskey, 1990)
Pupation - Late May - October

Appendix 2 A list of black fly species and the plants with which they have been associated.

Prosimulium hirtipes (= *mixtum /fuscum* and/ or *fontanum*)	
Prunus pennsylvanica (pin cherry)	Davies and Peterson, 1956
Vaccinium angustifolium (blueberry)	Davies and Peterson, 1956
Vaccinium myrtilloides (blueberry)	Davies and Peterson, 1956
Stegopterna mutata	
V. myrtilloides	Davies and Peterson, 1956
Simulium euryadminiculum	
willow (female shrub)	Davies and Peterson, 1956
S. vittatum	
V. myrtilloides	Davies and Peterson, 1956
Mixed flowers: yarrow, buckwheat,	Davies and Peterson, 1956
oxeye daisy, vetch, devil's paint brush	Davies and Peterson, 1956
and goldenrod	Davies and Peterson, 1956
S. decorum	
V. angustifolium	Davies and Peterson, 1956
V. myrtilloides	Davies and Peterson, 1956
S. furculatum	
Salix cordifolia (willow)	Hocking, 1953
Rubus acaulis (arctic raspberry)	Hocking and Pickering, 1954
Achillea millefolium (yarrow)	Hocking and Pickering, 1954
S. venustum	
Prunus pennsylvanica (pin cherry)	Davies and Peterson, 1956
V. angustifolium	Davies and Peterson, 1956
V. myrtilloides	Davies and Peterson, 1956
Cicuta mackenzieana (Parsely Fam.)	Hocking, 1953
Heracleum maximum (Parsely Fam.)	Hocking and Pickering, 1954
Ledum groenlandicum (labrador tea)	Hocking and Pickering, 1954
Rubus acaulis (arctic raspberry)	Hocking and Pickering, 1954
Achillea millefolium (yarrow)	Hocking and Pickering, 1954
S. pecuarum	
Salix interior (willow)	Robertson, 1928
Chaerophyllum procumbens	Robertson, 1928
Zizia aurea (golden alexander)	Robertson, 1928
Sassafras variifolium	Robertson, 1928
S. salopiensis	
Hedera helix (Ivy)	Smart, 1943
Simulium spp.	
Rubus acaulis (arctic raspberry)	Müller, 1873, *in* Proctor and Yeo, 1972
Achillea millefolium (yarrow)	Müller,, 1873, *in* Proctor and Yeo, 1972
Chrysosplenium alternifera	Müller, 1873, *in* Proctor and Yeo, 1972
Adoxa moschatellina (Moschatel)	Müller, 1873, *in* Proctor and Yeo, 1972
Salix viminalis (Osier)	Knuth, 1906-1909
Prunus serotina (rose family)	Robertson, 1928
Salix cordata (willow)	Robertson, 1928
Crataegus monogyna (Hawthorn)	Wenk, 1965
Hedera helix (Ivy)	Wenk, 1965
Pastinaca sativa (Wild Parsnip)	Wenk, 1965
Petroselium sativum (Umbelliferae)	Wenk, 1965
Polygonum aubertii (Milk Wart)	Wenk, 1965
Salix alba (White willow)	Wenk, 1965
Salix babylonica (Willow)	Wenk, 1965
Salix purpurea (Willow)	Wenk, 1965
Solidago canadensis (Goldenrod)	Wenk, 1965
Chrysosplenium spp. (Saxifrage)	Proctor and Yeo, 1972
Adoxa moschatellina (Moschatel)	Proctor and Yeo, 1972
Hedera helix (Ivy)	Proctor and Yeo, 1972
Malus sylvestris (Apple)	Proctor and Yeo, 1972
Salix viminalis (Osier)	Proctor and Yeo, 1972
Potentilla fruiticosa (shrubby cinqufoil)	Kearns, 1992

Appendix 3 List of Diptera considered to be sporadic pollinators of the blueberries *Vaccinium angustifolium* or *Vaccinium myrtilloides* in Nova Scotia.

Fam. Conopidae
- *Dalmannia nigriceps* Loew *
- *Myopa clausa* Loew *
- *Psithyrus fernaldae* Franklin
- *Psithyrus insularis* (Smith)

Fam. Bombylidae
- *Bombylius major* L.*
- *Bombylius pygmaeus pygmaeus* Fabricius *
- *Bombylius validus* Loew

Fam. Syrphidae
- *Syrphus autumnalis* Fluke
- *Syrphus ribesii* (L.)
- *Syrphus torvus* Osten Sacken
- *Parasyrphus sp.*
- *Dasysyrphus sp.*
- *Melangyna sp.*
- *Melanostoma mellinum* L. *
- *Meliscaeva cinctella* (Zetterstedt)
- *Shaerophoria abbreviata* Zetterstedt
- *Shaerophoria philanthus* (Meigen)
- *Shaerophoria scripta* L.*
- *Platycheirus inversus* Ide
- *Platycheirus peltatus* (Meigen)
- *Paragus sp.*
- *Chrysotoxum sp.*
- *Rhingia nasica* Say
- *Microdon sp.*
- *Volucella bombylans* (L.)
- *Sericomyia bifasciata* Williston *
- *Sericomyia chrysotoxoides* Macquart *
- *Sericomyia militaris* Walker
- *Sericomyia sexfasciacta* *
- *Sericomyia transversa* (Osburn)
- *Xylota annulifera* Bigot
- *Xylota hinei* (Curran)
- *Xylota vecors* Osten Sacken
- *Sphecomyia vittata* (Wiedemann)
- *Temnostoma vespiforme* (L.)
- *Eristalis anthophorina* (Fallen)
- *Eristalis armbustorum* L. *
- *Eristalis bastardi* Macquart *
- *Eristalis compactus* Walker *
- *Eristalis nemorum* (L.)
- *Eristalis rupium* Fabricius

All records from Finnamore and Neary (1978), except :
* From Phipps (1930)
FromSmall (1976)

Appendix 4 Studies in which the carbohydrates of phloem and honeydew were analysed.

Tarczynski et. al., 1992 (HPLC)

Bemisia tabaci	*Gossypium hirsutum* (cotton)
Honeydew carbohydrates	Phloem carbohydrates
- fructose	- sucrose
- sucrose	- raffinose series oligosac.;raffinose,
- glucose	- stachyose, verbascose and ajugose
- raffinose	- polyols; mannitol, sorbitol
- stachyose	
- trehalulose	

Byrne and Miller, 1990 (HPLC)

Bemisia tabaci	Poinsetta and Pumpkin	
Honeydew carbohydrates	Phloem carbohydrates	
- sucrose	- sucrose	- both
- glucose	- glucose	-both
- fructose	- fructose	- both
- stachyose	- stachyose	- pumpkin only
- raffinose	- raffinose	- pumpkin only
- galactose	- galactose	-pumpkin only
- melezitose		
- trehalulose		

Hussain et. al., 1974 (Paper chromatography)

myzus persicae (aphid)	*Raphanus sativus* (radish)
Honeydew carbohydrates	Phloem carbohydrates
- fructose	- fructose
- glucose	- glucose
- sucrose	
- trehalose	
- unknown oligosaccharides	
- melezitose	

Mittler, 1958 (Paper chromatography)

Tuberolachnus salignus (aphid)	*Salix actifolia* (willow)
Honeydew carbohydrates	Phloem carbohydrates
- sucrose	- sucrose
- fructose	
- glucose	
- melezitose	

Auclair, 1963 (Mostly by Paper Chromatography)

Additional accounts of melezitose presence in honeydew

Lachnus muravensis Arnhart	larch	
Lachnus robus L.	oak	(approx. 46 % of honeydew)
Lachnus pitchtae Mordwilko	fir	
Lecanium spp.	spruce	
aphid spp.	*Tilia spp.*	(approx. 40 % of honeydew)
aphid spp.	*Populs nigra*	(approx. 40 % of honeydew)
Tuberolachnus salignus (Gmelin)	willow	
Coccus hesperidium L.	lemon plants	
C. pseudomagnoliarum (Kuwana)	orange trees	
Eucallipterus tiliae L.	lime tree	
Planococcus citri (Risso)	lemon and grapefruit	
Saissetia oleae (Bernard)	orange tree	

Appendix 5 Carbohydrates which have been identified in black flies, sandflies, nectar (*Vaccinium angustifolium* and *V. myrtilloides*) and honeydew.

Sugar	Nectar	Black Fly	Sand Fly	Honeydew
Glucose	+	+	+	+
Fructose	+	+	+	+
Sucrose	+	+	+	+
Melibiose	—	+	—	+
Trehalose	—	—	+	+
Maltose	+	+	+	+
Turanose	—	—	+	+**
*Trehalulose	—	—	—	+**
*Erlose	—	—	+	+**
Maltotriose	—	—	—	+
Melezitose	—	—	+	+**
Raffinose	—	+	+	+
Stachyose	—	—	—	+

* not commercially available
** unique to honeydew
1) Nectar (*Vaccinium angustifolium* and *V. myrtilloides*) - (Free, 1970)
2) Black fly - (Lewis and Domoney, 1966; Watanabe, 1977)
3) Sand fly - (Moore *et al.,* 1987; MacVicker *et al.,* 1990; Wallbanks *et al.,* 1990)
4) Honeydew - (Auclair, 1963; Byrne and Miller, 1990; Tarczynski *et al.,* 1992)

Appendix 6 Various plant species identified from four habitats

Bog habitat:

- *Picea mariana* (Mill.) BSP (Black spruce)
- *Larix larcina* (Du Roi) Koch (Tamarack)
- *Chamaedaphne calyculata* (L.) Moench (Leather leaf)
- *Andromeda glaucophylla* Link (Bog rosemary)
- *Kalmia angustifolia L.* (Sheep laurel)
- *Kalmia polifolia* Wang. (Bog laurel)
- *Ledum groenlandicum* Oeder (Labrador tea)
- *Alnus rugosa* (Du Roi) Spreng. (Speckled alder)
- *Myrica gale* L. (Sweet gale)
- *Spiraea latifolia* (Ait.) Borkh. (Broad leaved meadowsweet)
- *Vaccinuim oxycoccus* L. (Small cranberry)

Air Field habitat:

- *Vaccinuim angustifolium* Ait. (Lowbush blueberry)
- *Vaccinium myrtilloides* Michx. (Velvet leaf blueberry)
- *Fragaria virginiana* Duchesne (Wild strawberry)
- *Taraxicum officinale* Weber (s.l.) (Common dandelion)
- *Amelanchier arborea* **?** (Michx.f.) Fern. (Canada serviceberry)
- *Hieracium aurantiacum* L. (Orange hawkweed)
- *Prunus pennsylvanica* L.f. (Pin cherry)
- *Comptonia peregrina* (L.) Coult. (Sweet fern)
- *Potentilla norvegica* L. (Rough cinquefoil)

Deciduous habitat:

- *Acer saccharum* Marsh. (Sugar maple)
- *Acer rubrum* L. (Red maple)
- *Fagus grandifolia* Ehrh. (Beech)
- *Betula papyrifera* Marsh. (White birch)
- *Populus tremuloides* Michx. (Trembling aspen)
- *Diervilla lonicera* Mill. (Bush honeysuckle)
- *Lonicera canadensis* Bartr. (Fly honeysuckle)
- *Aralia nudicaulis* L. (Wild sarsaparilla)
- *Smilacina recemosa* (L.) Desf. (False Solomon's seal)
- *Streptopus roseus* Michx. (Twisted stalk)
- *Cornus canadensis* L. (Bunchberry)
- *Vibernum alnifolium* Marsh. (Hobble bush)
- *Vibernum trilobum* Marsh. (High bush cranberry)
- *Erythronium americanum* Ker (Trout lily)

Coniferous habitat:

- *Pinus strobus* L. (White pine)
- *Pinus resinosa* Ait. (Red pine)
- *Picea glauca* (Moench) Voss (White spruce)
- *Abies balsamea* (L.) Mill. (Balsam fir)
- *Cornus canadensis* L. (Bunchberry)
- *Coptis groenlandica* (Oeder) Fassett (Gold thread)
- *Maianthemum canadensis* Desf. (Lily of the valley)
- *Clematis virginiana* L. (Virgin's bower)
- *Clintonia borealis* (Ait.) Raf. (Bluebeard lily)
- *Linnaea borealis* L. (Twin flower)
- *Trientilis borealis* Raf. (Star flower)
- *Polygala paucifolia* Willd. (Fringed polygala)
- *Galium triflorum* Michx. (Sweet scented bedstraw)
- *Mitella nuda* L. (Bishop's cap)
- *Gaultheria procumbens* L. (Wintergreen)

Appendix 7 Collection dates and times for tamarack samples, including the number of flies collected and the number used in T. L. C. analysis.

Date	Time	Conditions	No. Black Flies	No. T.L.C.[1]
June 02	7:30 PM	11 °C Sun	10 F 9 M	19
June 02	7:45 PM		21 F 7 M	28
June 12	7:30 PM	14 °C Sun	5 F 12 M	17
June 12	7:45 PM		63 F 7 M	70
June 16	7:07 PM	24 °C Sun, breeze	4 F 0 M	4
June 16	7:25 PM		0 F 1 M	1
June 17	7:30 PM	19 °C Cloud	5 F 3 M	8
June 17	7:46 PM		25 F 4 M	29
June 19	6:46 PM	19 °C Cloud	1 F 2 M	3
June 19	6:58 PM		1 F 1 M	2
June 24	7:18 PM	23 °C Sun	2 F 1 M	3
June 24	7:34 PM		5 F 0 M	5
June 26	7:18 PM	21 °C Sun	3 F 4 M	7
June 26	7:31 PM		13 F 1 M	14
June 28	7:48 PM	15 °C Sun	0 F 1 M	1
June 28	8:04 PM		0 F 0 M	0
July 06	7:46 PM	20 °C Sun	2 F 2 M	4
July 06	8:02 PM		5 F 1 M	6
			AM 32 F 34 M	221
			PM 133 F 22 M	
			165 F 56 M	

[1] 7 male and 13 female flies from June 02 were pooled and are not included in subsequent sugar profile analyses.

Appendix 8 Total numbers of male and female black flies from six species collected from a tamarack stand during early and late insect net sweeps from nine sample days.

	June								July	
Species*	02	12	16	17	19	24	26	28	06	Total
S. ven.	45	76	3	34	4	4	18	1	4	189
S. rost.	—	6	1	2	1	1	—	—	3	14
S. vit.	—	1	1	—	—	1	2	—	3	8
St. mut.	—	3	—	—	—	2	1	—	—	6
S. aur.	2	—	—	1	—	—	—	—	—	3
S. queb.	—	1	—	—	—	—	—	—	—	1
Total	47	87	5	37	5	8	21	1	10	221

* S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), St. mut. (*Stegopterna mutata*), S. aur. (*S. aureum*), and S. queb. (*S. quebecense*).

Appendix 9 Collection dates and times for car trap samples, including the number of flies collected and the number used in T. L. C. analyses using the Urea reagent

Date	Time	Conditions[1]	No. Black Flies		No. T.L.C.[2]
May 21	10:35 AM	13 °C Cloud	31 F	215 M	20
May 21	6:40 PM	11 °C Cloud	219 F	159 M	20
May 27	10:45 AM	12 °C Cloud	125 F	160 M	20
May 27	6:30 PM	13 °C Cloud	343 F	375 M	20
June 03	11:30 AM	11 °C Cloud	218 F	863 M	20
June 03	7:20 PM	10 °C Pt. Cloud	307 F	1020 M	20
June 10	11:20 AM	15 °C Pt. Cloud	182 F	237 M	20
June 10	6:52 PM	14 °C Cloud	230 F	312 M	20
June 16	11:00 AM	14 °C Sun	137 F	148 M	20
June 16	6:50 PM	16 °C Cloud	101 F	327 M	20
June 24	10:00 AM	20 °C Sun	186 F	282 M	20
June 24	7:20 PM	23 °C Sun	269 F	319 M	20
July 02	10:30 AM	14 °C Sun	106 F	229 M	20
July 02	7:15 PM	15 °C Sun	78 F	143 M	20
July 09	11:45 AM	26 °C Cloud	52 F	98 M	20
July 09	7:30 PM	24 °C Cloud, storm	63 F	115 M	20
		AM	1037 F	2232 M	320
		PM	1610 F	2770 M	
		Total	2647 F	5002 M	= 7649

1 Pt. = partial

2 An additional 10 male and 10 female flies from June 03 and 10 male and 10 female flies from June 16 were used in preliminary trials using D. A. P. A.

Appendix 10 Collection dates and times for Davies Bog samples, including the number of flies collected and the number used in T. L. C. analysis.

Date	Time	Conditions		No. Black Flies	No. T.L.C.
May 21	11:10 AM	14 °C Cloud		33 F	22
May 21	7:10 PM	9 °C Cloud		61 F	22
May 27	11:10 AM	14 °C Sun		133 F	22
May 27	7:48 PM	15 °C Cloud		202 F	22
June 03	11:49 AM	11 °C Cloud, drizzle		51 F, 1 M	22
June 03	7:52 PM	12 °C Sun		55 F	22
June 10	11:57 AM	16 °C Sun, breeze		116 F	22
June 10	7:40 PM	13 °C Sun		188 F	22
June 16	11:35 AM	14 °C Sun, breeze		67 F	22
June 16	7:08 PM	16 °C Cloud		212 F	22
June 24	10:27 AM	19 °C Sun		31 F	22
June 24	7:41 PM	24 °C Sun		7 F	7
July 02	11:06 AM	15 °C Sun		13 F	13
July 02	7:37 PM	17 °C Sun		11 F	11
July 09	11:56 AM	26 °C Cloud, breeze		12 F	12
July 09	8:04 PM	24 °C Cloud, drizzle		23 F	22
			AM	456 F, 1 M	307
			PM	759 F	
				1215 F, 1 M	

Appendix 11 Species composition of flies tested by T.L.C. from sweep net samples of four habitats on 8 sample days.

	Habitats				
Species	Davies Bog	Air Field	Deciduous Site	Coniferous Site	Tot.
S. ven.	168 (54.7)	150 (63.3)	59 (34.5)	33 (35.1)	410
S. rost.	41 (13.4)	44 (18.6)	7 (4.09)	5 (5.32)	97
S. tub.	10 (3.26)	13 (5.49)	2 (1.17)	6 (6.38)	31
S. aur.	2 (0.65)	6 (2.53)	1 (0.59)	5 (5.32)	14
S. queb.	—	1 (0.43)	1 (0.59)	21 (22.3)	23
S. decor.	—	5 (2.11)	1 (0.59)	1 (1.06)	7
S. vern.	1 (0.33)	—	2 (1.17)	8 (8.51)	11
S. parn.	3 (0.97)	1 (0.43)	—	1 (1.06)	5
S. eury.	46 (15.0)	7 (2.95)	—	6 (6.38)	59
S. vit.	34 (11.1)	9 (3.80)	4 (2.34)	—	47
P. fu. / mi.	2 (0.65)	1 (0.43)	6 (3.51)	—	9
St. mut.	—	—	63 (36.8)	5 (5.32)	68
P. font.	—	—	25 (14.6)	—	25
E. inven.	—	—	—	2 (2.13)	2
S. croxt.	—	—	—	1 (1.06)	1
Tot.	307	237	171	94	809

Species are as follows: S. ven. (*Simulium venustum*), S. rost. (*S. rostratum*), S. tub. (*S. tuberosum*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*), S. decor. (*S. decorum*), S. vern. (*S. vernum*), S. parn. (*S. parnasum*), S. eury. (*S. euryadminiculum*), S. vit. (*S. vittatum*), S. croxt. (*S. croxtoni)*, St. mut. (*Stegopterna mutata*), P. fu. / mi. (*Prosimulium fuscum / P. mixtum*), P. font. (*P. fontanum*), and E. inven. (*Ectemnia invenusta*).

Appendix 12 Dates on which various species of black fly were collected from the Davies Bog.

	May		June				July		
Species	21	27	03	10	16	24	02	09	Total
S. ven.	14	24	35	28	25	26	9	7	168
S. eury.	26	14	1	1	3	—	1	—	46
S. rost.	—	2	3	7	3	2	6	18	41
S. vit.	3	2	5	8	12	—	2	2	34
S. tub.	—	—	—	—	1	1	4	4	10
S. parn.	—	—	—	—	—	—	—	3	3
S. aur.	—	1	—	—	—	—	1	—	2
P. fu./mi.	1	1	—	—	—	—	—	—	2
S. vern.	—	—	—	—	—	—	1	—	1
Total	44	44	44	44	44	29	24	34	307

Species are as follows: S. ven. (*Simulium venustum*), S. rost. (*S. rostratum*), S. tub. (*S. tuberosum*), S. aur. (*S. aureum*), S. parn. (*S. parnasum*), S. eury. (*S. euryadminiculum*), S. vit. (*S. vittatum*), S. vern. (*S. vernum*), and P. fu. / mi. (*Prosimulium fuscum / P. mixtum*).

Appendix 13 Collection dates and times for air field samples, including the number of flies collected and the number used in T. L. C. analysis.

Date	Time	Conditions	No. Black Flies	No. T.L.C.
May 21	10:38 AM	14 °C Cloud, wind	4 F	4
May 21	6:37 PM	13 °C Cloud, wind	2 F	2
May 27	10:49 AM	15 °C Cloud	48 F, 1 M	22
May 27	6:26 PM	15 °C Cloud	101 F	22
June 03	11:28 AM	14 °C Cloud	17 F	17
June 03	7:20 PM	10 °C Cloud, breeze	13 F, 1 M	13
June 10	11:26 AM	17 °C Cloud	23 F	22
June 10	7:02 PM	15 °C Cloud, breeze	20 F	20
June 16	11:15 AM	15 °C Sun	33 F	22
June 16	6:47 PM	16 °C Cloud	49 F	22
June 24	10:10 AM	21 °C Sun	11 F	11
June 24	7:16 PM	25 °C Sun	9 F	9
July 02	10:38 AM	17 °C Sun	5 F	5
July 02	7:12 PM	17 °C Sun	8 F	8
July 09	11:35 AM	29 °C Cloud, breeze	16 F	16
July 09	7:43 PM	26 °C Cloud, drizzle	37 F	22
		AM	157 F, 2 M	237
		PM	239 F	
			396 F, 2 M	

Appendix 14 Dates on which various species of black fly were collected from the air field.

	May		June				July		
Species	21	27	03	10	16	24	02	09	Total
S. ven.	4	30	24	37	37	13	3	2	150
S. rost.	1	6	1	2	3	1	—	30	44
S. tub.	—	1	2	—	—	4	5	1	13
S. vit.	—	—	—	3	1	2	—	3	9
S. eury.	—	4	1	—	2	—	—	—	7
S. aur.	1	2	—	—	1	—	—	2	6
S. decor.	—	—	—	—	—	—	5	—	5
S. parn.	—	—	1	—	—	—	—	—	1
S. queb.	—	—	1	—	—	—	—	—	1
P. fu./mi.	—	1	—	—	—	—	—	—	1
Total	6	44	30	42	44	20	13	38	237

Species are as follows: S. ven. (*Simulium venustum*), S. rost. (*S. rostratum*), S. tub. (*S. tuberosum*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*), S. decor. (*S. decorum*), S. parn. (*S. parnasum*), S. eury. (*S. euryadminiculum*), S. vit. (*S. vittatum*), and P. fu. / mi. (*Prosimulium fuscum* / *P. mixtum*).

Appendix 15 Collection dates and times for deciduous habitat samples, including the number of flies collected and the number used in T. L. C. analysis.

Date	Time	Conditions	No. Black Flies	No. T.L.C.
May 21	10:48 AM	12 °C Cloud	26 F	22
May 21	6:55 PM	11 °C Cloud	9 F	9
May 27	11:19 AM	11 °C Cloud, breeze	39 F, 1 M	22
May 27	6:44 PM	13 °C Sun	25 F	22
June 03	11:45 AM	10 °C Cloud, drizzle	14 F	14
June 03	7:36 PM	10 °C Cloud	46 F	22
June 10	11:33 AM	13 °C Cloud	12 F	12
June 10	7:16 PM	13 °C Cloud	17 F	17
June 16	11:27 AM	14 °C Sun, breeze	0 F	0
June 16	6:57 PM	15 °C Cloud	0 F	0
June 24	10:26 AM	20 °C Sun	5 F	5
June 24	7:29 PM	21 °C Sun	4 F	4
July 02	10:48 AM	12 °C Sun	8 F	8
July 02	7:24 PM	13 °C Sun	7 F	7
July 09	11:48 AM	25 °C Cloud	2 F	2
July 09	7:57 PM	24 °C Cloud, drizzle	5 F	5
			AM 106 F, 1 M	171
			PM 113 F	
			219 F, 1 M	

Appendix 16 Dates on which various species of black fly were collected from the deciduous site.

	May		June				July		
Species	21	27	03	10	16	24	02	09	Total
St. mut.	22	12	21	7	—	1	—	—	63
S. ven.	3	16	3	15	—	6	13	3	59
P. font.	6	13	5	1	—	—	—	—	25
S. rost.	—	—	—	2	—	—	1	4	7
P. fu./mi.	—	2	3	1	—	—	—	—	6
S. vit.	—	—	4	—	—	—	—	—	4
S. tub.	—	—	—	1	—	—	1	—	2
S. vern.	—	—	—	1	—	1	—	—	2
S. queb.	—	—	—	—	—	1	—	—	1
S. decor.	—	—	—	1	—	—	—	—	1
S. aur.	—	1	—	—	—	—	—	—	1
Total	31	44	36	29	0	9	15	7	171

Species are as follows: S. ven. (*Simulium venustum*), S. rost. (*S. rostratum*), S. tub. (*S. tuberosum*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*), S. decor. (*S. decorum*), S. vern. (*S. vernum*), S. vit. (*S. vittatum*), St. mut. (*Stegopterna mutata*), and P. fu. / mi. (*Prosimulium fuscum / P. mixtum*) P font. (*P. fontanum*).

Appendix 17 Collection dates and times for coniferous habitat samples, including the number of flies collected and the number used in T. L. C. analysis.

Date	Time	Conditions	No. Black Flies	No. T.L.C.
May 21	10:18 AM	12 °C Cloud	3 F, 1 M	3
May 21	6:33 PM	10 °C Cloud	1 F	1
May 27	10:55 AM	10 °C Cloud, breeze	6 F	6
May 27	6:20 PM	11 °C Sun	6 F, 2 M	6
June 03	11:20 AM	10 °C Cloud	7 F	7
June 03	7:15 PM	10 °C Cloud, drizzle	3 F	3
June 10	11:17 AM	14 °C Cloud	5 F	5
June 10	6:50 PM	15 °C Cloud	3 F	3
June 16	11:08 AM	14 °C Sun	7 F	7
June 16	6:39 PM	16 °C Cloud	6 F	6
June 24	10:08 AM	19 °C Sun	6 F	6
June 24	7:12 PM	23 °C Sun	6 F	6
July 02	10:23 AM	13 °C Sun	8 F	8
July 02	6:58 PM	14 °C Sun	6 F	6
July 09	11:30 AM	24 °C Cloud	11 F	11
July 09	7:39 PM	23 °C Cloud	10 F	10
			AM 53 F, 1 M	94
			PM 41 F, 2 M	
			94 F, 3 M	

Appendix 18 Dates on which various species of black fly were collected from the coniferous site.

	May		June				July		
Species	21	27	03	10	16	24	02	09	Total
S. ven.	1	3	5	—	—	4	5	15	33
S. queb.	—	—	—	3	11	4	—	3	21
S. vern.	1	1	3	1	—	1	1	—	8
S. eury.	2	3	—	1	—	—	—	—	6
S. tub.	—	—	—	—	—	2	2	2	6
S. rost.	—	—	—	—	—	—	4	1	5
St. mut.	—	—	—	3	1	1	—	—	5
S. aur.	—	4	—	—	1	—	—	—	5
E. inven.	—	—	2	—	—	—	—	—	2
S. croxt.	—	1	—	—	—	—	—	—	1
S. parn.	—	—	—	—	—	—	1	—	1
S. decor.	—	—	—	—	—	—	1	—	1
Total	4	12	10	8	13	12	14	21	94

Species are as follows: S. ven. (*Simulium venustum*), S. rost. (*S. rostratum*), S. tub. (*S. tuberosum*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*), S. decor. (*S. decorum*), S. vern. (*S. vernum*), S. parn. (*S. parnasum*), S. eury. (*S. euryadminiculum*), S. croxt. (*S. croxtoni*), St. mut. (*Stegopterna mutata*), and E. inven. (*Ectemnia invenusta*).

Appendix 19 The hR_f values for standard sugars developed using the D. A. P. A. and urea reagents. ($hR_f = 100 \times R_f$).

Sugar	Standards hR_f mean (range) D. A. P. A.*	Urea**
Fru	58.5 (56.3 - 62.1)	60.2 (55.6 - 64.3)
Glc	54.1 (52.3 - 56.5)	-- --
Gal	50.0 (48.3 - 51.6)	-- --
Suc	47.0 (44.6 - 49.7)	47.6 (42.6 - 50.5)
Tur	45.7 (42.9 - 49.4)	45.0 (39.3 - 47.8)
Mal	37.0 (34.5 - 40.6)	-- --
Mez	33.5 (31.2 - 36.3)	35.8 (29.6 - 38.4)
Mel	30.9 (28.0 - 34.8)	-- --
Raf	26.0 (24.1 - 28.8)	27.4 (22.7 - 29.5)
Sta	13.0 (11.8 - 15.1)	13.8 (10.6 - 16.3)

* Means for each sugar based on measurement of 12 spots.
** Means for each sugar based on measurement of 12 spots.
The urea reagent does not react with the sugars indicated by the dashed lines.
Sugars are: Fru (fructose),Glc (glucose), Gal (galactose), Suc (sucrose), Tur (turanose), Mal (maltose), Mez (melezitose), Mel (melibiose), Raf (raffinose) and Sta (stachyose).

Appendix 20 hR_f values for sugars found in honeydew based on 6 samples taken on June 02 and June 17. Four samples from each date were developed using the D.A.P.A. reagent, and two samples from each date were developed using the Urea reagent. (hR_f = 100 x R_f).

D.A.P.A.

	June 02				June 17				
Sample	1	2	3	4	1	2	3	4	hR_f mean (range)
Fru	57.3	57.4		55.7	56.8	56.5	58.3	59.2	57.3 (55.7 - 59.2)
Glc	52.2	51.3		51.3	52.9	52.1	53.2	53.4	52.3 (51.3 - 53.4)
Suc/Tur	49.1	48.6				48.8			48.8 (48.6 - 49.1)
Raf	25.6			27.2		28.5			27.1 (25.6 - 28.5)
Sta	10.9	11.3	12.8	15.1	13.2	11.9	11.6		12.4 (10.9 - 15.1)

Urea*

	June 02		June 17		
Sample	5	6	5	6	hR_f mean (range)
Fru	59.8	60.2	60.4	60.5	60.2 (59.8 - 60.5)
Glc	——	——	——	——	——
Suc/Tur			47.3		47.3
Raf	26.5		26.9	26.2	26.5 (26.2 - 26.9)
Sta	11.9	12.3	12.6	12.4	12.3 (11.9 - 12.6)

* Glucose is not detected by the urea reagent.

Appendix 21 hR_f values for sugars found in *V. angustifolium* nectar based on 4 samples taken on June 03 and 3 samples taken on June 17. Samples from each date were developed using the D.A.P.A. and Urea reagents. ($hR_f = 100 \times R_f$).

	June 03				June 17				
	1	2	3	4	1	2	3	hR_f mean	(range)
D. A. P. A.									
Fru	56.9	60.7	59.9	58.2	58.6	59.2	60.3	59.1	(56.9 - 60.7)
Glc	52.4	55.2	53.8	53.8	54.5	53.3	54.8	54.0	(52.4 - 55.2)
Suc/Tur	—	—	45.8	43.6	44.1	—	—	44.5	(43.6 - 45.8)
Mal	—	41.1	39.7	—	39.0	39.2	—	39.8	(39.0 - 41.1)
UREA*									
Fru	57.3	56.8	58.5	57.0	56.9	59.4	59.5	57.9	(56.8 - 59.5)
Glc	—	—	—	—	—	—	—	—	—
Suc/Tur	—	—	45.2	44.8	44.3	—	—	44.8	(44.3 - 45.2)
Mal	—	—	—	—	—	—	—	—	—

* Glucose and maltose are not detected by the urea reagent.

Appendix 22: The hR_f values of sugars found in black fly gut contents developed using the D. A. P. A. reagent. ($hR_f = 100 \times R_f$)

Sugar	hR_f Mean (Range) June 03	n =	hR_f Mean (Range) June 16	n =
Fru	58.5 (55.7 - 61.9)	20	58.8 (56.4 - 60.8)	20
Glc	54.3 (52.6 - 55.9)	20	53.6 (52.0 - 56.1)	20
Gal	-- --	0	50.5 --	1
Suc/Tur	45.4 (41.6 - 47.3)	5	47.1 (43.6 - 49.0)	4
Mal	37.9 (37.4 - 38.3)	2	36.9 (36.7 - 37.0)	2
Mez	32.8 (29.6 - 34.5)	3	34.6 (31.5 - 36.1)	4
Mel	29.5 --	1	29.2 (29.1 - 29.3)	2
Raf	26.5 (26.0 - 28.3)	4	26.1 (24.3 - 27.9)	2
Sta	12.8 (12.7 - 12.9)	2	12.8 (11.0 - 14.7)	4

* 10 male and 10 female *S. venustum* / *S. rostratum* from June 03, and June 16 car trap samples were tested.
Sugars are: Fru (fructose),Glc (glucose), Gal (galactose), Suc (sucrose) / Tur (turanose), Mal (maltose), Mez (melezitose), Mel (melibiose), Raf (raffinose) and Sta (stachyose).

Appendix 23 The hR_f values of sugars found in black fly gut contents developed using the urea reagent. ($hR_f = 100 \times R_f$)

Sugar	hR_f Mean (Range) Urea*	
Fru	58.3	(55.4 - 62.3)
Suc / Tur	46.6	(42.3 - 49.1)
Mez	33.6	(29.2 - 36.1)
Raf	27.5	(22.3 - 32.8)
Sta	12.9	(10.2 - 15.8)

* Means for each sugar based on measurement of 12 spots. Sugars are: Fru (fructose),Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).

Appendix 24 Sugar combinations of male and female black flies collected during early sweep samples of a tamarack stand, on nine sample days.

		Combinations*																	
Date /Sex / Species		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
June 02																			
M	S. ven.	—	—	1	—	—	2	—	—	1	1	—	—	—	—	—	—	—	= 5
	S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
F	S. ven.	—	2	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	= 4
	S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 12																			
M	S. ven.	2	—	—	—	—	3	—	—	1	—	—	—	—	4	—	1	—	=11
	S. queb.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 1
F	S. ven.	—	—	—	—	—	1	—	—	1	—	—	—	—	2	—	—	—	= 4
	St. mut.	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	= 1
June 16																			
M	——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 0
F	S. ven.	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 2
	S. vit.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
	S. rost.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	= 1
June 17																			
M	S. ven.	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	= 3
F	S. ven.	—	3	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 4
	S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 19																			
M	S. ven.	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 2
F	S. rost.	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	= 1
June 24																			
M	S. vit.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
F	S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
	St. mut.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
June 26																			
M	S. ven.	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 4
F	S. ven.	—	1	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	= 3
June 28																			
M	S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
F	——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 0
July 06																			
M	S. ven.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 1
	S. vit.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
F	S. ven.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	= 2
Total		3	17	3	0	0	18	1	0	5	1	0	1	0	6	1	2	0	=58

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), St. mut. (*Stegopterna mutata*), S. aur. (*S. aureum*), and S. queb. (*S. quebecense*).

Note: 3 male and 5 female *S. venustum* from June 02 were not included as these samples were pooled

Appendix 25 Sugar combinations of male and female black flies collected during late sweep samples of a tamarack stand, on nine sample days.

Date /Sex / Species		Combinations*																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
June 02																			
M	S. ven.	—	1	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	= 3
F	S. ven.	—	5	2	—	—	4	—	—	2	—	—	—	—	—	—	—	—	= 13
June 12																			
M	S. ven.	—	—	—	—	—	2	—	—	—	1	—	—	—	3	—	—	1	= 7
F	S. ven.	—	18	7	—	3	3	6	2	6	1	—	—	1	5	2	—	—	= 54
	S. rost.	—	1	2	—	—	—	1	1	—	—	—	—	1	—	—	—	—	= 6
	St. mut.	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
	S. vit.	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 1
June 16																			
M	S. ven.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 1
F	——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 0
June 17																			
M	S. ven.	—	—	1	—	—	2	—	—	1	—	—	—	—	—	—	—	—	= 4
F	S. ven.	2	8	2	—	—	6	1	—	1	—	—	—	—	1	1	1	—	= 23
	S. rost.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	= 1
	S. aur.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 19																			
M	S. ven.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	= 1
F	S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 24																			
M	——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 0
F	S. ven.	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
	S. rost.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
	St. mut.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 26																			
M	S. ven.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	= 1
F	S. ven.	1	6	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	= 10
	S. vit.	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 2
	St. mut.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 28																			
M	——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 0
F	——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 0
July 06																			
M	S. rost.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 1
F	S. ven.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
	S. rost.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
	S. vit.	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	= 2
	Total	4	45	20	2	3	23	10	3	10	3	1	0	2	10	3	3	1	= 143

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), St. mut. (*Stegopterna mutata*) and S. aur. (*S. aureum*).

Note: 4 male and 8 female *S. venustum* from June 02 were not included as these samples were pooled.

Appendix 26 Sugar combinations in crop and midgut of *S.venustum* and four other species from tamarack samples.

Combination	Number (Percent)		
	S. venustum	Other* Species	Total
(1) Fru, Glc	53 (32.5)	9 (29.0)	62 (32.0)
(2) Fru, Glc, Suc/Tur	17 (10.4)	6 (19.4)	23 (11.8)
(3) Fru, Glc, Mez	— —	2 (6.5)	2 (1.0)
(4) Fru, Glc, Raf	3 (1.8)	— —	3 (1.6)
(5) Fru, Glc, Sta	38 (23.3)	3 (9.7)	41 (21.1)
(6) Fru, Glc, Suc/Tur, Mez	7 (4.3)	4 (12.9)	11 (5.7)
(7) Fru, Glc, Suc/Tur, Raf	2 (1.2)	1 (3.2)	3 (1.6)
(8) Fru, Glc, Suc/Tur, Sta	14 (8.6)	1 (3.2)	15 (7.7)
(9) Fru, Glc, Raf, Sta	4 (2.5)	— —	4 (2.1)
(10) Fru, Glc, Suc/Tur, Mez, Raf	— —	1 (3.2)	1 (0.005)
(11) Fru, Glc, Suc/Tur, Mez, Sta	— —	1 (3.2)	1 (0.005)
(12) Fru, Glc, Mez, Raf, Sta	1 (0.006)	1 (3.5)	2 (1.0)
(13) Fru, Glc, Suc/Tur, Raf, Sta	15 (9.2)	1 (3.2)	16 (8.3)
(14) Fru, Glc, Suc/Tur, Mez, Raf, Sta	4 (2.5)	— —	4 (2.1)
(15) Sta	4 (2.5)	1 (3.2)	5 (2.6)
(16) Raf, Sta	1 (0.006)	— —	1 (0.005)
Total	163	31	194

Sugars are: Fru (fructose), Glc (glucose), Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).
Note: Does not include 13 female and 7 male flies used in preliminary trials. Also does not include 7 individuals containing no sugars.
* Other species includes: *S. rostratum, S. vittatum*, *S. aureum*, *S. quebecense* and *St. mutata*.

Appendix 27 Sugar combination of male and female *S. venustum** collected during AM and PM car trap samples, on eight sample days.

		Combinations**																	
Sex / Date		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
	AM																		
Male	May 21	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	May 27	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	June 03	—	2	1	1	—	—	—	—	—	—	4	1	—	—	1	—	—	= 10
	June 10	—	2	1	—	—	—	6	—	—	—	—	—	—	—	1	—	—	= 10
	June 16	—	5	1	—	—	—	3	—	—	—	1	—	—	—	—	—	—	= 10
	June 24	—	6	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	July 02	—	7	1	—	—	—	—	1	—	—	1	—	—	—	—	—	—	= 10
	July 09	—	3	2	—	—	—	1	1	—	—	2	—	—	—	1	—	—	= 10
	MaleTotal	0	45	10	1	0	0	10	2	0	0	8	1	0	0	3	0	0	= 80
Female	May 21	—	7	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	May 27	—	—	—	—	—	—	8	—	—	—	2	—	—	—	—	—	—	= 10
	June 03	—	3	—	1	3	—	1	—	—	—	1	—	—	—	1	—	—	= 10
	June 10	—	1	1	—	—	1	2	—	2	—	2	—	1	—	—	—	—	= 10
	June 16	—	5	2	—	—	—	1	—	—	—	—	2	—	—	—	—	—	= 10
	June 24	—	4	3	—	—	—	2	—	—	—	—	1	—	—	—	—	—	= 10
	July 02	—	4	1	—	—	—	4	—	—	—	—	1	—	—	—	—	—	= 10
	July 09	—	3	2	—	—	—	1	2	—	—	1	—	—	1	—	—	—	= 10
	Female Total	0	27	12	1	3	1	19	2	2	0	6	4	1	1	1	0	0	= 80
AM Total			72	22	2	3	1	29	4	2	0	14	5	1	1	4	0	0	= 160
	PM																		
Male	May 21	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	May 27	—	9	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	June 03	—	8	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	= 10
	June 10	—	2	—	—	—	—	4	3	—	—	1	—	—	—	—	—	—	= 10
	June 16	—	4	5	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 10
	June 24	—	3	1	1	—	—	5	—	—	—	—	—	—	—	—	—	—	= 10
	July 02	—	3	3	—	1	—	—	—	—	1	—	2	—	—	—	—	—	= 10
	July 09	—	5	1	—	—	—	1	—	—	1	1	—	—	—	1	—	—	= 10
	Male Total	0	44	11	1	1	0	11	3	0	2	4	2	0	0	1	0	0	= 80
Female	May 21	—	9	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	May 27	—	3	1	—	—	—	5	—	—	—	1	—	—	—	—	—	—	= 10
	June 03	—	6	—	—	—	—	3	—	—	—	1	—	—	—	—	—	—	= 10
	June 10	—	2	2	—	—	—	2	—	—	1	—	3	—	—	—	—	—	= 10
	June 16	—	4	4	—	2	—	—	—	—	—	—	—	—	—	—	—	—	= 10
	June 24	—	2	—	—	1	—	2	4	—	—	1	—	—	—	—	—	—	= 10
	July 02	—	—	4	—	—	—	1	2	—	—	—	—	—	2	1	—	—	= 10
	July 09	—	1	1	—	1	—	2	2	—	—	1	—	—	2	—	—	—	= 10
	Female Total	0	27	13	0	4	0	15	8	0	1	4	3	0	4	1	0	0	= 80
PM Total			71	24	1	5	0	26	11	0	3	8	5	0	4	2	0	0	= 160
Overall Total			143	46	3	8	1	55	15	2	3	22	10	1	5	6	0	0	= 320

* Some *S. rostratum* may be present

** The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

Appendix 28 Sugar combinations in crop and midgut of *S. venustum* [1] from car trap samples.

Combination [2]	Number (Percent)		
	Female	Male	Total
(1) Fru, Glc	54 (33.8)	89 (55.6)	143 (44.7)
(2) Fru, Glc, Suc/Tur	25 (15.6)	21 (13.1)	46 (14.4)
(3) Fru, Glc, Mez	1 (0.6)	2 (1.3)	3 (0.9)
(4) Fru, Glc, Raf	7 (4.4)	1 (0.6)	8 (2.5)
(5) Fru, Glc, Sta	1 (0.6)	— —	1 (0.3)
(6) Fru, Glc, Suc/Tur, Mez	34 (21.3)	21 (13.1)	55 (17.2)
(7) Fru, Glc, Suc/Tur, Raf	10 (6.3)	5 (3.1)	15 (4.7)
(8) Fru, Glc, Suc/Tur, Sta	2 (1.3)	— —	2 (0.6)
(9) Fru, Glc, Raf, Sta	1 (0.6)	2 (1.3)	3 (0.9)
(10) Fru, Glc, Suc/Tur, Mez, Raf	10 (6.3)	12 (7.5)	22 (6.9)
(11) Fru, Glc, Suc/Tur, Mez, Sta	7 (4.4)	3 (1.9)	10 (3.1)
(12) Fru, Glc, Mez, Raf, Sta	1 (0.6)	— —	1 (0.3)
(13) Fru, Glc, Suc/Tur, Raf, Sta	5 (3.1)	— —	5 (1.6)
(14) Fru, Glc, Suc/Tur, Mez, Raf, Sta	2 (1.3)	4 (2.5)	6 (1.9)
(15) Sta	— —	— —	— —
(16) Raf, Sta	— —	— —	— —
Total	160	160	320

[1] Some *S. rostratum* may be present

[2] Sugars are: Fru (fructose), Glc (glucose), Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).

Appendix 29 Sugar combinations of female black flies collected during AM sweep samples of Davies Bog habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
S. ven.	3	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 6
S. eury.	1	7	4	2	—	—	—	1	—	—	—	—	—	—	—	—	—	=15
P. fu/mi.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
May 27																		
S. ven.	—	7	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	=10
S. eury.	—	3	7	—	—	—	1	—	—	—	—	—	—	—	—	—	—	=11
S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
S. ven.	—	8	3	—	—	2	1	2	—	—	—	—	—	—	—	—	—	=16
S. vit.	—	—	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	= 3
S. rost.	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 2
S. eury.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
June 10																		
S. ven.	—	5	2	—	—	4	2	—	—	1	—	—	—	—	—	—	—	=14
S. vit.	—	—	1	1	—	—	—	1	—	—	1	—	—	—	—	—	—	= 4
S. rost.	—	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. eury.	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 16																		
S. ven.	3	7	—	—	—	—	1	—	—	—	—	—	—	2	—	—	—	=13
S. vit.	—	1	3	1	—	—	1	1	—	—	1	—	—	—	—	—	—	= 8
S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 24																		
S. ven.	—	11	2	—	—	1	—	1	1	—	—	—	—	2	1	—	—	=19
S. rost.	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. tub.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 1
July 02																		
S. ven.	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. tub.	—	—	1	—	—	2	1	—	—	—	—	—	—	—	—	—	—	= 4
S. rost.	—	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 3
S. vit.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. vern.	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. eury.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 09																		
S. ven.	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. rost.	—	—	2	—	—	—	—	1	1	—	1	—	—	—	—	—	—	= 5
S. tub.	—	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 4
Total	8	62	37	7	2	12	11	7	2	1	3	0	0	4	1	0	0	= 157

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), S. aur. (*S. aureum*), S. tub. (*S. tuberosum*), S. eury. (*S. euryadminiculum*), S. vern. (*S. vernum*), and P. fu/mi. (*Prosimulium fuscum / mixtum*).

Appendix 30 Sugar combinations of female black flies collected during PM sweep samples of Davies Bog habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
S. ven.	3	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 8
S. eury.	1	4	4	—	—	—	1	1	—	—	—	—	—	—	—	—	—	=11
S. vit.	—	2	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 3
May 27																		
S. ven.	—	6	3	2	—	—	3	—	—	—	—	—	—	—	—	—	—	=14
S. eury.	—	1	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	= 3
S. rost.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. vit.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
P. fu/mi.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
S. ven.	—	7	1	2	1	4	3	1	—	—	—	—	—	—	—	—	—	=19
S. vit.	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	= 2
S. rost.	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	= 1
June 10																		
S. ven.	—	1	1	3	2	3	—	1	—	2	—	1	—	—	—	—	—	=14
S. rost.	—	—	3	—	—	—	—	—	—	—	—	1	—	—	—	—	—	= 4
S. vit.	—	—	1	2	—	1	—	—	—	—	—	—	—	—	—	—	—	= 4
June 16																		
S. ven.	1	4	2	—	—	3	2	—	—	—	—	—	—	—	—	—	—	=12
S. vit.	—	1	—	—	—	—	1	—	—	—	1	1	—	—	—	—	—	= 4
S. eury.	—	—	1	—	—	—	—	—	—	—	—	2	—	—	—	—	—	= 3
S. tub.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. rost.	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	= 2
June 24																		
S. ven.	—	2	2	—	—	1	—	1	—	—	—	—	1	—	—	—	—	= 7
July 02																		
S. ven.	—	—	3	1	—	2	—	1	—	—	—	—	—	—	—	—	—	= 7
S. rost.	—	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. vit.	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	= 1
July 09																		
S. ven.	—	2	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 4
S. rost.	—	4	1	1	—	—	2	2	—	—	2	—	—	—	1	—	—	=13
S. parn.	—	2	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 3
S. vit.	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 2
Total	5	43	30	12	3	18	14	10	0	2	5	5	1	1	1	0	0	= 150

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), S. tub. (*S. tuberosum*), S. eury. (*S. euryadminiculum*), S. parn. (*S. parnasum*), and P. fu/mi. (*Prosimulium fuscum / mixtum*).

Appendix 31 Sugar combinations in crop and midgut contents, of *S.venustum* and other species from Davies Bog samples.

Combination	Number (Percent)		
	S.venustum	Other spp.*	Total
(1) Fru, Glc	67 (43.0)	38 (27.5)	105 (35.7)
(2) Fru, Glc, Suc/Tur	26 (16.7)	41 (29.7)	67 (22.8)
(3) Fru, Glc, Mez	9 (5.8)	10 (7.3)	19 (6.5)
(4) Fru, Glc, Raf	3 (1.9)	2 (1.5)	5 (1.7)
(5) Fru, Glc, Sta	20 (12.8)	10 (7.3)	30 (10.2)
(6) Fru, Glc, Suc/Tur, Mez	13 (8.3)	2 (8.7)	25 (8.5)
(7) Fru, Glc, Suc/Tur, Raf	7 (4.5)	10 (7.3)	17 (5.9)
(8) Fru, Glc, Suc/Tur, Sta	1 (0.6)	1 (0.7)	2 (0.7)
(9) Fru, Glc, Raf, Sta	3 (1.9)	— —	3 (1.0)
(10) Fru, Glc, Suc/Tur, Mez, Raf	1 (0.6)	7 (5.1)	8 (2.7)
(11) Fru, Glc, Suc/Tur, Mez, Sta	1 (0.6)	4 (2.9)	5 (1.7)
(12) Fru, Glc, Mez, Raf, Sta	1 (0.6)	— —	1 (0.3)
(13) Fru, Glc, Suc/Tur, Raf, Sta	4 (2.6)	1 (0.7)	5 (1.7)
(14) Fru, Glc, Suc/Tur, Mez, Raf, Sta	1 (0.6)	1 (0.7)	2 (0.7)
(15) Sta	— —	— —	— —
(16) Raf, Sta	— —	— —	— —
Total	156	138	294

Sugars are: Fru (fructose), Glc (glucose), Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).
No sugars were detected in 7 *S. venustum* and 1 *S. euryadminiculum* in AM samples and 4 *S. venustum* and 1 *S. euryadminiculum* from PM samples.
Other species includes: *S. rostratum*, *S. tuberosum*, *S. aureum*, *S. vernum*, *S. parnasum*, *S. euryadminiculum*, *S. vittatum* and *Prosimulium fuscum / mixtum*

Appendix 32 Sugar combinations of female black flies collected during AM sweep samples of the air field habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
S. ven.	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
May 27																		
S. ven.	—	11	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	=14
S. rost.	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 4
S. aur.	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. tub.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. eury.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
S. ven.	1	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	=14
S. tub.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 10																		
S. ven.	—	13	4	—	—	—	2	—	—	—	—	—	—	1	1	—	—	=21
S. rost.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 16																		
S. ven.	—	9	2	—	—	1	2	—	1	—	1	—	—	1	2	—	—	=19
S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. eury.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
June 24																		
S. ven.	1	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 5
S. tub.	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. vit.	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	= 2
S. rost.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	= 1
July 02																		
S. decor.	1	1	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 5
July 09																		
S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. rost.	—	4	2	—	—	—	—	2	—	—	—	2	—	1	1	—	—	=12
S. aur.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. tub.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	= 1
S. vit.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
Total	3	75	15	2	0	1	8	2	1	0	1	2	0	4	5	0	0	= 119

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), S. tub. (*S. tuberosum*), S. eury. (*S. euryadminiculum*), S. aur. (*S. aureum*), and S. dec. (*S. decorum*).

Appendix 33 Sugar combinations of female black flies collected during PM sweep samples of the air field habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
S. ven.	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
May 27																		
S. ven.	1	8	1	1	—	—	5	—	—	—	—	—	—	—	—	—	—	=16
S. eury.	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 3
S. rost.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
P. fu/mi.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
S. ven.	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	=10
S. queb.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. eury.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
E. parn.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 10																		
S. ven.	—	10	4	—	—	—	—	—	—	—	—	2	—	—	—	—	—	=16
S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. vit.	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
June 16																		
S. ven.	3	9	3	—	—	—	2	—	—	—	1	—	—	—	—	—	—	=18
S. rost.	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. vit.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. eury.	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	= 1
June 24																		
S. ven.	—	3	3	—	—	—	—	—	—	—	—	—	—	—	2	—	—	= 8
S. tub.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 02																		
S. ven.	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. tub.	—	3	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	= 5
July 09																		
S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. rost.	1	2	8	—	1	—	—	1	—	—	—	—	—	3	2	—	—	=18
S. vit.	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	= 2
S. aur.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	= 1
Total	6	59	27	1	1	0	9	1	0	0	2	4	0	4	4	0	0	= 118

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*), S. tub. (*S. tuberosum*), S. eury. (*S. euryadminiculum*), S. parn. (*S. parnasum*) and P. fu/mi. (*Prosimulium fuscum / mixtum*).

Appendix 34 Sugar combinations in crop and midgut contents, of *S.venustum* and other species from air field samples.

Combination	Number (Percent)		
	S.venustum	Other spp.	Total
(1) Fru, Glc	96 (66.7)	38 (45.2)	134 (58.8)
(2) Fru, Glc, Suc/Tur	21 (14.6)	21 (25.0)	42 (18.4)
(3) Fru, Glc, Mez	3 (2.1)	— —	3 (1.3)
(4) Fru, Glc, Raf	— —	1 (1.2)	1 (0.4)
(5) Fru, Glc, Sta	1 (0.7)	— —	1 (0.4)
(6) Fru, Glc, Suc/Tur, Mez	11 (7.6)	6 (7.1)	17 (7.5)
(7) Fru, Glc, Suc/Tur, Raf	— —	3 (3.6)	3 (1.3)
(8) Fru, Glc, Suc/Tur, Sta	1 (0.7)	— —	1 (0.4)
(9) Fru, Glc, Raf, Sta	— —	— —	— —
(10) Fru, Glc, Suc/Tur, Mez, Raf	2 (1.4)	1 (1.2)	3 (1.3)
(11) Fru, Glc, Suc/Tur, Mez, Sta	2 (1.4)	4 (4.8)	6 (2.6)
(12) Fru, Glc, Mez, Raf, Sta	— —	— —	— —
(13) Fru, Glc, Suc/Tur, Raf, Sta	2 (1.4)	6 (7.1)	8 (3.5)
(14) Fru, Glc, Suc/Tur, Mez, Raf, Sta	5 (3.5)	4 (4.8)	9 (4.0)
(15) Sta	— —	— —	— —
(16) Raf, Sta	— —	— —	— —
Total	144	84	228

Sugars are: Fru (fructose), Glc (glucose), Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).
No sugars were detected in 2 *S. venustum* / *S. rostratum* and 1 *S. decorum* from AM samples and 6 *S. venustum* / *S. rostratum* from PM samples.
Other species includes: *S. rostratum*, *S. tuberosum*, *S. aureum*, *S. quebecense*, *S. decorum*, *S. parnasum*, *S. euryadminiculum*, *S. vittatum* and *Prosimulium fuscum* / *mixtum*.

Appendix 35 Sugar combinations of female black flies collected during AM sweep samples of the deciduous habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
S. mut.	—	7	4	—	—	—	1	—	—	—	—	4	—	—	—	—	—	=16
P. font.	—	2	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 4
S. ven.	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
May 27																		
S. ven.	—	4	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	= 9
St. mut.	3	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	=10
P. font.	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	= 1
P. fu/mi.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. aur.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
S. ven.	—	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	= 3
St. mut.	1	2	1	—	—	—	5	—	—	—	—	—	—	—	—	—	—	= 9
P. font.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
P. fu/mi.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 10																		
S. ven.	—	5	2	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 8
St. mut.	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 2
S. tub.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. vern.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 16																		
——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	=—
June 24																		
S. ven.	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	—	—	= 3
S. vern.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. queb.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 02																		
S. ven.	—	2	—	—	1	—	4	1	—	—	—	—	—	—	—	—	—	= 8
July 09																		
S. rost.	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	= 2
Total	7	34	16	2	1	0	14	3	0	0	2	4	0	2	0	0	0	=85

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. tub. (*S. tuberosum*), St. mut. (*Stegopterna mutata*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*) S. vern. (*S. vernum*), P. font. (*Prosimulium fontanum*), and P. fu/mi (*P. fuscum / mixtum*).

Appendix 36 Sugar combinations of female black flies collected during PM sweep samples of the deciduous habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
St. mut.	—	5	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 6
P. font.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. ven.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
May 27																		
S. ven.	—	2	1	—	—	—	4	—	—	—	—	—	—	—	—	—	—	= 7
St. mut.	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
P. font.	1	3	2	1	—	—	5	—	—	—	—	—	—	—	—	—	—	=12
P. fu/mi.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
St. mut.	1	7	1	—	—	—	3	—	—	—	—	—	—	—	—	—	—	=12
P. font.	—	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 4
P. fu/mi.	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 2
S. vit.	—	1	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	= 4
June 10																		
S. ven.	—	5	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	= 7
St. mut.	—	3	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	= 5
S. rost.	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	= 2
P. font.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
P. fu/mi.	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	= 1
S. decor.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 16																		
——	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= —
June 24																		
S. ven.	—	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	= 3
St. mut.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 02																		
S. ven.	—	3	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	= 5
S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. tub.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 09																		
S. ven.	—	—	—	—	—	—	—	1	—	—	—	1	—	—	1	—	—	= 3
S. rost.	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	= 2
Total	4	41	11	4	0	1	15	1	1	0	0	6	0	1	1	0	0	=86

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vit. (*S. vittatum*), St. mut. (*Stegopterna mutata*), S. tub. (*S. tuberosum*), S. dec. (*S. decorum*), P. font. (*Prosimulium fontanum*), and P. fu/mi (*P. fuscum / mixtum*).

Appendix 37 Sugar combinations in crop and midgut of *S. venustum* and other species from deciduous samples.

Combination	Number (Percent)					
	S.venustum		Other spp.		Total	
(1) Fru, Glc	23	(39.7)	52	(51.0)	75	(46.9)
(2) Fru, Glc, Suc/Tur	11	(19.0)	16	(15.7)	27	(16.9)
(3) Fru, Glc, Mez	2	(3.5)	4	(3.9)	6	(3.8)
(4) Fru, Glc, Raf	1	(1.7)	—	—	1	(0.6)
(5) Fru, Glc, Sta	1	(1.7)	—	—	1	(0.6)
(6) Fru, Glc, Suc/Tur, Mez	12	(20.7)	17	(16.7)	29	(18.1)
(7) Fru, Glc, Suc/Tur, Raf	2	(3.5)	2	(2.0)	4	(2.5)
(8) Fru, Glc, Suc/Tur, Sta	—	—	1	(1.0)	1	(0.6)
(9) Fru, Glc, Raf, Sta	—	—	—	—	—	—
(10) Fru, Glc, Suc/Tur, Mez, Raf	1	(1.7)	1	(1.0)	2	(1.3)
(11) Fru, Glc, Suc/Tur, Mez, Sta	2	(3.5)	8	(7.8)	10	(6.3)
(12) Fru, Glc, Mez, Raf, Sta	—	—	—	—	—	—
(13) Fru, Glc, Suc/Tur, Raf, Sta	2	(3.5)	1	(1.0)	3	(1.9)
(14) Fru, Glc, Suc/Tur, Mez, Raf, Sta	1	(1.7)	—	—	1	(0.6)
(15) Sta	—	—	—	—	—	—
(16) Raf, Sta	—	—	—	—	—	—
Total	58		102		160	

Sugars are: Fru (fructose), Glc (glucose), Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).
No sugars were detected in 4 *St. mutata*, 1 *S. venustum* / *S. rostratum*, 1 *S. aureum* and 1 *S. vernum* from AM samples and 3 *St. mutata* and 1 *Prosimulium fontanum* from PM samples.
Other species includes: *S. rostratum*, *S. tuberosum*, *S. aureum*, *S. quebecense*, S. *decorum*, *S. vernum*, *S. vittatum*, *Prosimulium fuscum* / *mixtum*, *P. fontanum* and *St. mutata*.

Appendix 38 Sugar combinations of female black flies collected during AM sweep samples of the coniferous habitat, on eight sample days.

Date / Species	Combinations*																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																		
S. eury.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
S. vern.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
May 27																		
S. aur.	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 4
S. crox.	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																		
S. ven.	1	2	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	= 5
S. vern.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
E. inv.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 10																		
S. queb.	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. vern.	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 1
St. mut.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 16																		
S. queb.	1	2	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 5
St. mut.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. aur.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
June 24																		
S. queb.	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 2
S. ven.	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 2
S. tub.	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	= 2
July 02																		
S. ven.	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 3
S. rost.	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
S. tub.	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
S. dec.	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 1
July 09																		
S. ven.	—	2	2	—	—	—	1	—	—	1	—	—	—	—	—	—	—	= 6
S. queb.	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	—	—	= 3
S. tub.	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 2
Total	3	20	13	2	0	0	6	0	1	1	5	0	0	2	0	0	0	= 53

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vern. (*S. vernum*), St. mut. (*Stegopterna mutata*), S. aur. (*S. aureum*), S. queb. (*S. quebecense*), S. tub. (*S. tuberosum*), S. crox. (*S. croxtoni*), S. dec. (*S. decorum*), S. eury. (*S. euryadminiculum*) and E. inv. (*Ectemnia invenusta*).

Appendix 39 Sugar combinations of female black flies collected during PM sweep samples of the coniferous habitat, on eight sample days.

		Combinations*																	
Date / Species		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
May 21																			
	S. ven.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
May 27																			
	S. eury.	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	= 3
	S. ven.	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
	S. vern.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 03																			
	S. vern.	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
	E. inv.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
June 10																			
	St. mut.	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	= 2
	S. eury.	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 1
June 16																			
	S. queb.	—	3	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	= 6
June 24																			
	S. ven.	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 2
	S. queb.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
	S. vern.	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	= 1
	St. mut.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 02																			
	S. ven.	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 2
	S. rost.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
	S. vern.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
	S. parn.	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	= 1
	S. tub.	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	= 1
July 09																			
	S. ven.	—	7	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	= 9
	S. rost.	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	= 1
Total		0	23	5	1	0	0	6	2	0	0	4	0	0	0	0	0	0	=41

* The sugar combinations are as follows: **(0)** No Sugars, **(1)** Fru, Glc, **(2)** Fru, Glc, Suc/Tur, **(3)** Fru, Glc, Mez, **(4)** Fru, Glc, Raf, **(5)** Fru, Glc, Sta, **(6)** Fru, Glc, Suc/Tur, Mez, **(7)** Fru, Glc, Suc/Tur, Raf, **(8)** Fru, Glc, Suc/Tur, Sta, **(9)** Fru, Glc, Raf, Sta, **(10)** Fru, Glc, Suc/Tur, Mez, Raf, **(11)** Fru, Glc, Suc/Tur, Mez, Sta, **(12)** Fru, Glc, Mez, Raf, Sta, **(13)** Fru, Glc, Suc/Tur, Raf, Sta, **(14)** Fru, Glc, Suc/Tur, Mez, Raf, Sta, **(15)** Sta, **(16)** Raf, Sta.

** The species are as follows: S. ven. (*Simulium venustum*) , S. rost. (*S. rostratum*), S. vern. (*S. vernum*), St. mut. (*Stegopterna mutata*), S. eury. (*S. euryadminiculum*) S. queb. (*S. quebecense*), S. tub. (*S. tuberosum*), S. parn. (*S. parnasum*), and E. inv. (*Ectemnia invenusta*).

Appendix 40 Sugar combinations in crop and midgut of *S. venustum* and other species from coniferous samples.

Combination	Number (Percent)		
	S.venustum	Other spp.	Total
(1) Fru, Glc	19 (59.4)	24 (40.7)	43 (47.3)
(2) Fru, Glc, Suc/Tur	5 (15.6)	13 (22.0)	18 (19.8)
(3) Fru, Glc, Mez	1 (3.1)	2 (3.4)	3 (3.3)
(4) Fru, Glc, Raf	— —	— —	— —
(5) Fru, Glc, Sta	— —	— —	— —
(6) Fru, Glc, Suc/Tur, Mez	3 (9.4)	9 (15.3)	12 (13.2)
(7) Fru, Glc, Suc/Tur, Raf	— —	2 (3.4)	2 (2.2)
(8) Fru, Glc, Suc/Tur, Sta	— —	1 (1.7)	1 (1.1)
(9) Fru, Glc, Raf, Sta	1 (3.1)	— —	1 (1.1)
(10) Fru, Glc, Suc/Tur, Mez, Raf	3 (9.4)	6 (10.2)	9 (9.9)
(11) Fru, Glc, Suc/Tur, Mez, Sta	— —	— —	— —
(12) Fru, Glc, Mez, Raf, Sta	— —	— —	— —
(13) Fru, Glc, Suc/Tur, Raf, Sta	— —	2 (3.4)	2 (2.2)
(14) Fru, Glc, Suc/Tur, Mez, Raf, Sta	— —	— —	— —
(15) Sta	— —	— —	— —
(16) Raf, Sta	— —	— —	— —
Total	32	59	91

Sugars are: Fru (fructose), Glc (glucose), Suc (sucrose) / Tur (turanose), Mez (melezitose), Raf (raffinose) and Sta (stachyose).
No sugars were detected in 1 *S. venustum* / *S. rostratum*, 1 *S. quebecense* and 1 *S. tuberosum* from AM samples. Sugars were detected in all flies from PM samples.
Other species includes: *S. rostratum*, *S. tuberosum*, *S. aureum*, *S. quebecense*, *S. decorum*, *S. vernum*, *S. parnasum*, *S. euryadminiculum*, *S. croxtoni*, *St. mutata* and *Ectemnia invenusta*.